ENDOSCOPIC IMAGING AND IMAGE-GUIDED SAMPLING FOR PANCREATIC NEOPLASIA

by

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Abstract

Background: Endoscopic ultrasound (EUS) provides high-resolution images of the pancreas. There are two fundamental echoendoscope designs, yet no data support use of one type over the other. Once lesion is detected, EUS-guided fine-needle aspiration (FNA) is performed, but standard (STN) processing techniques can be associated with limited cellularity, leading to evaluation of novel filter-clot (FC) cellblock technique. With improved quality of EUS-FNA samples, it may be possible to pre-operatively evaluate biomarker status of pancreatic lesions to better risk stratify and tailor future treatments to individual patients. Aims: 1) To compare pancreatic lesion detection rates using radial and linear EUS and to determine incremental diagnostic yield of second EUS exam in tandem study. 2) To compare diagnostic yield and accuracy of STN and FC techniques of processing EUS-FNA samples. 3) To determine whether DPC4 gene status using EUS-FNA samples correlate with clinical outcomes in pancreatic cancer (PC) patients.

Methods: High-risk individuals (HRIs) in screening program underwent radial or linear EUS or tandem radial and linear EUS in randomized order. Pancreatic lesion detection rates were compared. EUS-FNA samples during a 13-month period were used to compare the sample adequacy and diagnostic accuracy of STN and FC techniques. Retrospective review was performed evaluating whether Dpc4 immunolabeling status in EUS-FNA samples of PC patients correlated with pattern of disease progression and clinical outcomes.

Results: In HRIs, linear EUS detected more pancreatic lesions than radial EUS. In tandem EUS exams, second EUS detected additional lesions, whether the initial exam was performed with radial or linear EUS. However, incremental detection rate was significantly higher if the second exam was linear EUS. FC technique had higher sample adequacy and diagnostic accuracy than STN technique. DPC4 status was associated with pattern of failure, with DPC4 negative PC more likely to progress with metastasis.

Conclusion: Linear EUS should be the echoendoscope of choice in pancreatic imaging. FC technique was associated with improved quality of EUS-FNA samples. DPC4 status correlated with disease progression phenotypes in PC patients. Pre-operative biomarker studies using EUS-FNA samples have the potential to advance the concept of personalized medicine in the field of pancreatic oncology.

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Table of Contents

page

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

List of Figures

Chapter 2

Chapter 3

Chapter 4

Chapter 1: Background/Introduction

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Pancreatic Cancer: Why Consider Screening?

Pancreatic cancer remains one of the most deadly diseases, despite significant advances in medicine over the past decade. Pancreatic adenocarcinoma is the fourth leading cause of cancer deaths in the United States for both males and females with an estimated 44,030 new cases and 37,660 deaths in 2011.[1] In contrast to the other leading causes of cancer death (lung, colorectal, breast, and prostate) which has shown a decline since 2003, the death rate from pancreatic adenocarcinoma has increased during the same time period.[1] Unfortunately, majority of the symptomatic patients are incurable. The prognosis for patients with pancreatic adenocarcinoma remains poor, with 5-year relative survival rate of only 6% for all stages combined likely due to the late stage of disease at the time of diagnosis. Hence, there has been strong interest in detecting precursor lesions or small asymptomatic cancers which are potentially curable. Widespread screening program does not seem feasible or cost-effective given the relative low incidence of the disease, accounting for only 3% of all new cancer cases in the United States, and the lack of accurate, inexpensive, and non-invasive diagnostic tests for early lesions. However, screening may be desirable in selected population with increased risk for developing pancreatic adenocarcinoma.

Genetic Predisposition to Pancreatic Cancer

Although the great majority of pancreatic adenocarcinoma cases are thought to be sporadic in nature, it has been estimated that up to 10% of cases can be attributed to genetic factors. [2-4] In fact, familial clustering of pancreatic cancer was noted as early as 1967 when Lynch reported on an adenocarcinoma-prone family.[5] Familial pancreatic cancer (FPC) is characterized by two or more first-degree relatives with pancreatic adenocarcinoma in the absence of a known cancer syndromes or other diseases with known genetic defect. Individuals from a family with a pair of affected first-degree relatives have a higher risk (6.4-fold to 32-fold) of developing pancreatic cancer.[6-9] Thus far, the key causative gene or genes leading to the inherited predisposition in familial pancreatic cancer have not yet been fully elucidated. Complex segregation analysis suggests that this predisposition may be due to a novel rare major gene with an autosomal dominant inheritance with reduced penetrance.[10-13]

Initial linkage analysis suggested that the palladin gene may be one of the FPC gene where a base pair change appeared to track with the development of pancreatic cancer in the specific kindred.[14] However, the initial excitement has been tempered by the failure of population-based studies in Canada and Europe to demonstrate that mutations in the PALLD gene are more common in those with FPC compared to controls.[15-18] Furthermore, a study evaluating the pattern of palladin protein expression in 177 cases of pancreatic adenocarcinoma determined that while the palladin protein is over-expressed in the stroma, it is not over-expressed in the neoplastic cells in pancreatic cancer.[19]

To date, germline BRCA2 mutation appears to be the most common genetic abnormality in patients who develop pancreatic adenocarcinoma from familial pancreatic cancer kindreds, but still have only been reported in 6-19% of all FPC kindreds.[20-22] Mutations in the BRCA2 gene can be present even when in the absence of breast or ovarian cancer, and in apparently sporadic pancreatic cancer. Recent studies have identified a another associated inheritable gene mutation called PALB2 (partner and localizer for breast cancer 2 gene) as a pancreatic adenocarcinoma susceptibility gene, which may also be causative for 3-4% of FPC.[7, 9] The PALB2 protein directly binds BRCA1 and acts as a bridge between BRCA1 and BRCA2 to form a complex involved in double-strand break repair.[23] The PALB2 gene is present in 1-2% of famililal breast cancer. Subsequent testing of patients with a personal history of breast and pancreatic cancer[24] and non-BRCA1/2 breast cancer women with a personal or family history of pancreatic cancer[25] have shown the PALB2 mutation to be very uncommon mutation. The clinical utility of routine testing of FPC patients for PALB2 has not been proven.

Inherited Cancer Syndromes

Hereditary Pancreatitis

Hereditary pancreatitis is a rare inherited disorder characterized by recurrent attacks of acute pancreatitis in childhood or early adolescence, followed by development of chronic pancreatitis in late adolescence or early adulthood.[26] It is

transmitted as an autosomal dominant disorder with incomplete penetrance.[27] Most are due to germline gain-of-function mutations in a cationic trypsinogen gene (PRSS1) on chromosome 7q35.[28-30] Mutations in PRSS1 result in premature trypsin activation and ineffective autodegradation of active trypsin mutants, leading to autodigestion and acute pancreatitis.[31] Hereditary pancreatitis is associated with one of the highest estimated lifetime risks for developing pancreatic cancer among the inherited pancreatic cancer syndrome, with lifetime risk approaching 40%. [32, 33] Particularly in those individuals with a paternal inheritance pattern, the cumulative risk for developing pancreatic cancer is approximately 75%.[32] Tobacco smoking increases the risk even further in this population by approximately 2-fold and decreases the age of onset of pancreatic cancer by approximately 20 years.[27, 34]

Peutz-Jeghers Syndrome (PJS)

Peutz-Jeghers syndrome (PJS) is an autosomal dominantly inherited polyposis syndrome with high penetrance. The reported frequency of PJS is 1 in 8300 to 280,000 individuals.[35] It is characterized by hamartomatous polyps of the gastrointestinal (GI)tract and mucocutaneous pigmentation. It is caused by inherited germline mutation of the STK11/LKB1 tumor suppressor gene.[36] Patients with PJS have a significantly increased lifetime risk for multiple GI cancers, including esophagus (0.5%), stomach (29%), small intestine (13%), and colon (39%).[37] They are also at increased risk for non-GI cancers, including breast (54%), lung (15%), ovaries (21%), cervix (10%), uterus (9%),

and testicles (9%). The cumulative lifetime risk for developing pancreatic cancer is 36%, with relative risk of 132.[37]

Familial Atypical Multiple Mole Melanoma (FAMMM)

Familial atypical multiple mole melanoma (FAMMM) is an autosomally dominant disease with variable penetrance. It is characterized by familial occurrence of multiple benign melanocytic nevi, dysplastic nevi, and melanoma.[38] It is associated with germline mutations in the p16/CDKN2A gene.[39, 40] In addition to pancreatic cancer, FAMMM is associated with an increased risk of sarcomas, endometrial, breast, and lung cancers.[41, 42] There is approximately 13-fold to 22-fold increased risk of pancreatic cancer in FAMMM compared to the general population.[42, 43]

Lynch Syndrome

Patients with hereditary non-polyposis colorectal cancer syndrome (HNPCC or Lynch Syndrome) have mutations in the mismatch repair genes (MLH1, MSH2, MSH6, and PMS2). It is characterized by early-onset colorectal cancer. They are also prone to develop other types of cancers, including endometrial, gastric, renal, ureteral, and small intestinal cancers.[44] Lifetime risk of pancreatic cancer in patients with Lynch syndrome is 3.7% up to the age of 70, which is an 8.6-fold increased risk compared to the general population.[45]

Familial Breast-Ovarian Cancer (FBOC)

Familial breast-ovarian cancer syndrome is an autosomal dominantly inherited syndrome associated with germline mutations in BRCA1 (breast related cancer 1) and BRCA2 (breast related cancer 2) tumor suppressor genes involved in repair of DNA damage. Carriers of the gene mutations are at a high risk for developing early-onset breast and/or ovarian cancers, as well as cancers of the gallbladder and bile duct (RR 4.97), prostate (RR 4.65), stomach (RR 2.59) and malignant melanoma (RR 2.58).[46] BRCA1 mutation is associated with a 2.3-fold to 3.6-fold increased risk [47, 48] and BRCA2 mutation is associated with a 3-fold to 10-fold increased risk for pancreatic cancer.[46, 49, 50] Furthermore, in patients with sporadic pancreatic cancer, 7.3% had germline BRCA2 mutation.[51] Approximately 1% of the general Ashkenazi Jewish population carry each of the BRCA1 and BRCA2 founder mutations.[52, 53] Studies have shown that in patients of Ashekenazi Jewish descent with pancreatic adenocarcinoma, 5.5-10% of them will have BRCA2 mutation.[52-55]

Targets for Screening and Surveillance

The ideal screening strategy for pancreatic cancer would target high grade benign, noninvasive precursor neoplastic lesions (pancreatic intraepithelial neoplasias called PanINs or intraductal papillary mucinous neoplasms called IPMNs) before malignant transformation or disease at an early stage which would allow for curative surgical resection.[56] Although IPMNs can be detected as cystic lesions and/or a dilated main pancreatic duct, PanINs are small branch ducts < 5 mm in size, often microscopic, and

not reliably visualized by clinical imaging tests. Hence, the optimal strategy for detection of early pancreatic neoplasia may need to involve biomarker tests alone or in combination with imaging.

Available and Anticipated Tumor Markers

Currently, there is no biomarker with adequate sensitivity and specificity which can be used for routine clinical screening.[57] Given the typical late stage of disease at the time of diagnosis, there has been much effort invested in identifying accurate tumor markers to aid in early diagnosis of pancreatic cancer.

The most widely used serum marker in patients with pancreatic cancer is sialylated Lewis blood group antigen on MUC-1 (Mucin 1, cell surface associated), carbohydrate antigen 19-9 (CA 19-9). It is a cell surface glycoprotein expressed by pancreatic cancer cells, but is also found in normal pancreatic and biliary duct cells, and gastric colonic, endometrial, and salivary epithelia.[58] Consequently, CA 19-9 is not routinely used for diagnosis because of the unacceptably high rate of false-positive results, with specificity ranging from 33% to 100%.[59-61]

CA 19-9 is also associated with imperfect sensitivity, ranging from 41% to 86%.[59, 61] Approximately 4-15% of the general population do not express Lewis antigen and therefore do not have detectable CA 19-9 levels.[61-65] In patients with resectable

pancreatic cancer, CA 19-9 level is elevated in only 65% of these patients.[61] The marker is also inadequate to reliably differentiate between pancreatic cancer and chronic pancreatitis, as up to 40% of patient with chronic pancreatitis can exhibit elevated levels of CA 19-9.[61, 66] Given its performance characteristics as a biomarker in the general population, serum CA 19-9 is used primarily for monitoring of responses to therapy in patients already diagnosed with cancer rather than for early diagnosis.[61, 67-69] One recent feasibility study in individuals with one or more first-degree relative with pancreatic cancer used serum CA19-9 as a screening test followed by EUS in those with elevated levels and detected one asymptomatic pancreatic ductal adenocarcinom (PDA) in 546 individuals[70].

Carcinoembryonic antigen (CEA) was the first biomarker used for diagnostics. Several studies have demonstrated high levels of CEA in the pancreatic juice of patients with pancreatic cancer compared to those with benign pancreatic disease.[71-74] When the CEA cut-off level was set at 50ng/mL, the positive predictive value (PPV), negative predictive value (NPV), and accuracy for diagnosing pancreatic cancer were 77%, 95%, and 85%, respectively.[71, 75] The main limitation of CEA is the low sensitivity, ranging from 25%-56% with relatively high specificity, ranging from 82%-100% in distinguishing pancreatic cancer from benign pancreatic diseases.[59, 76-81]

Much of the initial efforts in identifying novel markers of pancreatic cancer have focused on carbohydrate antigens of MUC-1 in hopes of improving the performance of CA 19-9.

PAM4 is an anti-MUC1 monoclonal antibody which appears to detect MUC-1 expressed by pancreatic cancer MUC-1 protein more specifically than MUC-1 proteins from other cancers (e.g., breast, ovarian, etc).[82] Furthermore, in comparison with CA 19-9, PAM4 demonstrated higher sensitivity and specificity in discriminating patients with pancreatic cancer from those with chronic pancreatitis ($p<0.003$).[82] As expected, patients with advanced disease had significantly higher levels that those with early disease. Diagnostic sensitivity of PAM4 for stage 3/stage 4 disease was 91%, for stage 2 was 86%, and for stage 1 was 62% (stage 1A 54% and stage 1B 75%).[83] Further supporting the potential role of PAM4 in detecting early-stage pancreatic cancer, PAM4 expression was detected in precursor lesions of pancreatic adenocarcinoma, positive in 89% of pancreatic intraepithelial neoplasias (PanIN) and 86% of intraductal papillary mucinous neoplasms (IMPN) examined, including 94% of the earliest neoplastic lesions, PanIN-1A and 1B.[84]

Recent studies have identified other potential biomarkers for pancreatic cancer, including CA494,[85] CEACAM1,[86] PTHrP,[87] TuM2-PK,[88] CAM 17.1,[78] and serum beta HCG.[89] Although their performance characteristics in initial studies are promising, larger studies are needed to confirm their clinical applicability and are currently used only in research setting.

Pancreatic juice sample provides a rich medium for genetic and epigenetic marker analysis. Pancreatic juice samples can be obtained at the time of EUS (secretin-

stimulated) or ERCP (duodenal aspirate[90] or pure pancreatic juice).[57] Markers which have been studied in pancreatic juice include K-ras mutations, p53 mutations, DNA methylation aberrations, and mitochondrial DNA mutations.[61] Mutant K-ras is a marker of interest because mutations are present in 90% of pancreatic adenocarcinoma and has been measured in pancreatic juice samples. However, its sensitivity and specificity for pancreatic cancer are poor (sensitivity 38-62%; specificity 88-90%), likely due to the fact mutant K-ras can also be found in chronic pancreatitis and in PanINs without pancreatic cancer.[57, 90-96] p53 mutations are found in approximately 70% of invasive pancreatic adenocarcinoma[91] and has been detected in 40-50% of pancreatic juice samples and brush cytology specimens with patients with pancreatic cancer.[97] DNA promoter methylation alterations has been investigated in multiple candidate genes, including p16,[98, 99] RELN,[100] DAB1,[100] ppENK,[101, 102] Cyclin D2,[103] SOCS1,[104] SPARC,[105] TSLC1,[106] and others.[61, 102, 107] DNA promoter hypermethylation status was quantified in a panel of candidate genes (Cyclin D2, FOXE1, NPTX2, ppENK, p16, and TFP12) in pure pancreatic juice obtained from patients with pancreatic ductal adenocarcinoma, intraductal papillary mucinous neoplasms, chronic pancreatitis and controls with no known pancreatic disease, as well as a cohort of high-risk individuals from familial pancreatic cancer kindreds. This method demonstrated high sensitivity (82%) and specificity (100%) in identifying patients with pancreatic cancer.[108] Mitochondrial DNA mutations are commonly found in multiple cancers.[61, 109-113] Using chip technologies, initial studies appear to suggest that mitochondrial mutations can be reliably detected in pancreatic juice samples from patients with pancreatic cancer.[61, 111]

Approaches to Screening

Currently, there is no sufficiently sensitive, specific, and reliable screening test for the early detection of pancreatic cancer. The great majority of pancreatic cancers are considered sporadic, accounting for at least 90% of all patients. The detection rate is low in average risk individuals because pancreatic cancer is a rare disease, despite its deadly nature. In screening studies performed in Japan, 5 cancers were found in 2511 individuals. Given the overall low incidence of disease and the current lack of accurate, inexpensive, and non-invasive screening tests, the consensus is that widespread population-based screening for pancreatic cancer in the general population or those with only one affected first-degree relative is neither feasible nor indicated in most countries.[56] Selective screening of patients with increased risk for pancreatic ductal adenocarcinoma has been performed in high risk patients from familial pancreatic cancer kindreds and patients with inherited cancer syndromes.[56, 114, 115]

The various approaches to screening and results of screening tests for asymptomatic pancreatic neoplasms are summarized in Table 1. One approach is population-based screening, such as that performed in Japan with abdominal ultrasound (with[199] or without[116] MRI). A second approach is the use of a serum biomarker, such as serum CA19-9 followed by a pancreatic imaging test[70]. A third approach uses only abdominal imaging tests, such as computed tomography, magnetic resonance imaging, endoscopic

ultrasonography (EUS), or endoscopic retrograde cholangiopancreatography (ERCP), in combination or in sequence (i.e. EUS following MRI/MRCP or CT if abnormal).

Multi-detector computed tomography (MDCT) is currently the abdominal imaging test of choice for pancreatic disease, particularly for diagnosis of solid tumors and staging of pancreatic cancer.[117, 118] Despite the high accuracy for detecting and staging of pancreatic malignancies, the sensitivity of MDCT may be suboptimal and may still miss lesion when used for screening for early pancreatic neoplasia.[114, 115, 118] Sensitivity of thin section triple phase helical CT to detect lesions smaller than 2cm was only 70-80%.[56, 119] Recent studies have shown that MDCT has a negative predictive value of 87% for tumor respectability[120] and an accuracy rate of 85-95%.[75, 121, 122] Furthermore, there is also a concern for radiation exposure if CT is used as a part of a long-term screening/surveillance program, particularly in individuals with impaired DNA mismatch repair gene function due to BRCA1/2 or PALB2 gene mutation. Hence, CT scan is not the ideal screening or surveillance imaging test for high risk individuals. Furthermore, MDCP with a pancreatic protocol may not be as sensitive as EUS in at-risk individuals from FPC kindreds[114, 115, 123, 124].

Magnetic resonance imaging (MRI) may be an interesting choice for non-invasive imaging test for screening high-risk patient because it is able to image the entire abdomen and pelvis (unlike EUS) while avoiding the radiation risk (unlike CT). Magnetic resonance cholangiopancreatography (MRCP) is able to non-invasively image pancreatic

ductal anatomy (unlike ERCP) and small cystic lesions, such as IPMNs. Preliminary data from high-risk patients who underwent surgical resection suggests that MRI/MRCP may be superior to CT, particularly for detection of IPMNs (71% versus 14%, p<0.0001).[56, 124] A prospective MRI-based screening study of 79 patients aged 39-72 with a p16 Leiden mutation (associated with FAMMM syndrome) has shown that early-stage pancreatic cancers can be detected at baseline and during follow-up[125]. After a median follow-up period of 4 years (range, 0-10 years), pancreatic cancer was diagnosed in 7 patients (9%). The mean age at diagnosis was 59 years (range, 49-72 years). Three of the asymptomatic pancreatic cancers were present at the first examination, and 4 were detected after a negative result in the initial examination. All 7 patients with cancer had a resectable lesion but 5 underwent surgery (3 had an R0 resection, and 2 had lymph node metastases). Furthermore, possible precursor lesions (ie, duct ectasias or branch-duct IPMNs, based on MRCP) were found in 9 individuals (11%).

Endoscopic ultrasound (EUS) has been used to screen high-risk individuals in several screening programs.[60, 114, 115, 126, 127] It can provide high-resolution images of the pancreas without the risk of radiation exposure and can image mural nodules (focal thickening of the wall in branch duct IMPNs), which are associated with increased risk of malignancy. [57, 118, 128] The disadvantage of EUS is that it is operator-dependent and is an endoscopic procedure with inherent risk of procedure and sedation, which may limit its role in widespread screening and surveillance program. Preliminary analysis of high-risk individuals enrolled in a screening program who underwent surgical resection suggest

that EUS can detect almost twice as many neoplastic lesions as CT or MRI/MRCP[56, 124]. Published studies using EUS-based screening for high risk individuals have reported detection of asymtptomatic precancerous branch duct IPMNs, large PanIns, incidental pancreatic endocrine tumors, and ductal adenocarcinomas. One Dutch study of BRCA1/2 and p16 germline mutation carriers, patients with Peutz-Jeghers syndrome, and relatives of patients reported the high one-time yield of EUS-based screening. The authors found a 6.8% prevalence (n=3 of 44 individuals screened) of asymptomatic pancreatic ductal adenocarcinomas (12, 20, and 50 mm in size)[126]. All cancers were completely resected but 2 already had lymph node metastases at presentation. Furthermore, the diagnostic yield of EUS-based screening for prevalent precursor branch duct IPMNs was 16%[126].

The clinical utility of ancillary studies such as fine needle aspiration (FNA) and endoscopic retrograde cholangiopancreatography (ERCP) is not clear. EUS-FNA has been used to investigate pancreatic cystic lesions and can provide a cytologic diagnosis of IPMN in 71% of the cases.[129] The need for routine FNA of pancreatic cysts in a high risk population has not been proven, given the typically small size of branch duct IPMNs (comprising the vast majority of cystic lesions) detected not requiring surgical treatment. EUS-FNA can also lead to false positive results if cytological aspirates show severe dysplasia or findings suspicious for ductal adenocarcinoma leading to potentially unnecessary surgery[114]. ERCP has been used routinely in high risk patients from FPC relatives with abnormal EUS, but this resulted in a post-ERCP pancreatitis rate of 7%[114]

in one study. Furthermore, ERCP did not reliably demonstrate ductal communication of branch duct IPMNs or lead to additional clinically-relevant imaging findings. Hence, most formal screening programs around the world do not recommend routine ERCP for asymptomatic individuals.

In summary, there is accumulating data that clinically available abdominal imaging tests such as EUS and MRI/MRCP can detect asymptomatic precursor benign (IPMN, PanIN) and invasive malignant pancreatic neoplasms such as ductal adenoarcinoma in individuals with an inherited predisposition. These asymptomatic FPCs detected have been more likely to be resectable, compared to symptomatic tumors. The most challenging part of screening high risk individuals is selection of individuals with high grade precursor neoplasms for preventive treatment (i.e. surgical resection prior to development of invasive cancer). Ongoing and future research should focus on formulating and validating a predictive model for FPC risk and neoplastic progression using patient characteristics, imaging, and biomarkers. The comparative cost and effectiveness of various approaches for screening and surveillance of high risk individuals also deserves study. For now, screening is best performed in high risk individuals within research protocols in academic centers with multidisciplinary teams with expertise in genetics, gastroenterology, radiology, surgery, and pathology.

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Table 1: Approaches to Pancreatic Screening

	Sequential Serum biomarker + Imaging	Sequential Abdominal Imaging Tests	Single or Concurrent Imaging Tests
Sporadic population		Transabdominal US followed by non-contrast MRI/MRCP, prospective study in 2511 patient found 5 cancers (4 resectable)[130]	Transabdominal US in 130,951 patients found 3 PDA[116]
High risk population	Serum CA19-9 followed by EUS if > 37 U/ml detected in 546 individuals with >= 1 FDR with PC found pancreatic neoplasms in 5/546 (1 early cancer)	MRI/MRCP or CT followed by EUS in FPC relatives found IPMN and PDA in 8.3%[131]	MRI/MRCP only in 79 p16 mutation carriers detected 5 cancers[125]
		EUS followed by ERCP (when	EUS + MRI/MRCP in FPC relatives

		abnormal) detected PanINs[60]	detected IPMNs and/or PDA[132], [123]
			EUS + MDCT in FPD relatives detected IPMNs, PNET, and PDA[114, 115]
			EUS only in FPC relatives and mutation carriers detected IPMNs and PDA[126]

Chapter 2: Radial versus Linear and Second Look Endoscopic Ultrasound (EUS) Improves Detection of Pancreatic Lesions: A Randomized Tandem Study

Introduction:

Endoscopic ultrasonography (EUS) is increasingly used as a diagnostic test for imaging pancreatobiliary abnormalities. In particular, when evaluating the pancreas for early neoplastic changes or lesions, magnetic resonance imaging (MRI) and EUS appear to be better imaging modalities than computed tomography (CT) in detecting small and early lesions, especially in asymptomatic high-risk individuals (HRIs) undergoing screening program.[1-8]

There are two fundamental types of echoendoscopes in clinical practice today, radial and linear array echoendoscope.[9, 10] Radial array echoendoscopes produce ultrasound images perpendicular to the axis of the endoscope tip with 360 degrees scanning range. The circumferential images produced are oriented similar to that of the cross-sectional CT and are often easier to interpret for a novice endosonographer. Radial EUS is used most often in diagnostic examination as the orientation of the transducers limit ultrasound-guided biopsy and therapeutic interventions. Linear array echoendoscopes produce ultrasound images parallel to the direction of insertion of the endoscope, with scanning range between 100 and 180 degrees. Linear array echoendoscopes offer a distinct advantage over the radial array echoendoscopes since fine needle aspiration (FNA) and therapeutic interventions are able to be performed through the instrument channel under real-time guidance.

Linear EUS is currently favored by many endosonographers for pancreatic imaging, regardless of whether or not FNA is required.[11, 12] However, no studies support the systematic use of linear over radial EUS for detection of non-malignant pancreatic lesions. Published data directly comparing radial and linear EUS have evaluated its role in diagnosing and staging pancreatic malignancies [13-17] and in diagnosing chronic pancreatitis with variable outcomes.[18, 19] In the published paper and abstract addressing the performance characteristics of EUS in non-malignant pancreatic imaging, radial and linear array EUS were found to be comparable in the diagnosis of chronic pancreatitis by EUS criteria.[18,19]

In the field of screening colonoscopy and adenoma detection, pooled results from tandem colonoscopy studies have shown a 22% miss rate for colon adenoma detection.[20-29] However, in the field of pancreatic diagnostic imaging, the miss rate for EUS detection of pancreatic lesions is unknown.

The primary aims of the study were to compare the pancreatic lesion detection rates using radial array and linear array EUS in a well-defined, asymptomatic high risk population undergoing pancreatic screening and to determine the incremental diagnostic yield of a second EUS exam in a randomized, tandem study.

Methods:

Study design:

This was a cohort study with embedded randomized tandem study as part of the multi-center American Cancer of the Pancreas Screening (CAPS 3) Consortium study, involving 5 academic centers. Endoscopic ultrasound (EUS) was performed as a part of the baseline screening evaluation by expert endosonographers at each participating institution on an outpatient basis. The institutional review boards of all 5 participating institutions approved the research protocol.

Study population:

Consecutive asymptomatic high-risk individuals (HRI) who consented to be part of the CAPS 3 study and underwent an endoscopic ultrasound examination at 1 of the 5 participating centers (Johns Hopkins Hospital (Baltimore, Maryland), Mayo Clinic (Rochester, Minnesota), University of California (Los Angeles, California), Dana Farber Cancer Institute (Boston, Massachusetts), and MD Anderson Cancer Center (Houston, Texas)) were screened for eligibility. HRIs included those with Peutz–Jeghers syndrome (PJS), familial breast-ovarian cancer patients with at least 1 affected first- or second-degree relative with pancreatic ductal adenocarcinoma (PC), and relatives of patients with familial pancreatic cancer (FPC) with at least 1 affected first-degree relative. Exclusion criteria included: inability to provide informed consent, prior pancreas screening, Karnofsky performance status of less than 60, any suspicion of pancreatic disease, prior pancreas surgery, severe medical illness, bleeding diathesis or thrombocytopenia, renal insufficiency, allergic reaction to radiographic contrast

material, morbid obesity, severe claustrophobia, and upper gastrointestinal tract obstruction.

Endoscopic ultrasound:

Advanced endoscopists with expertise in EUS performed all of the procedures. EUS imaging was performed using a mechanical or electronic radial array (GFUM20, GFUE160-AL5; Olympus Corporation, Center Valley, PA) and/or a linear array echoendoscope (CFUC140P, SSD-Alpha5 or Alpha10; Olympus Corporation). While some HRI had only one radial or linear EUS examinations, the majority of the participants underwent tandem radial and linear EUS in a randomized order. Random allocation sequence was performed using a random-numbers table and sequentially numbered, opaque, sealed envelopes at each site.

Pancreatic lesion was defined as a cystic lesion, a solid lesion, or a dilated main pancreatic duct. The size and location of each lesion were identified on a pancreas map for precise documentation for each EUS examination (Appendix 3).

Statistical methods:

The chi-square, Fisher exact test, t test, and Wilcoxon rank-sum test were performed for categoric and numeric variables, where appropriate, to compare characteristics. Two-tailed P values less than 0.05 were considered statistically significant. All statistical

analyses were performed using the Stata software package, version 11 (Stata Corp, College Station, Texas).

Results:

Table 1 summarizes the baseline characteristics of the high-risk individuals (HRIs) included in the study. A total of 278 asymptomatic HRIs were enrolled with mean age of 56.1 years (range 25.6-86.5 years), over 70% of whom were over the age of 50. The HRIs were predominantly Caucasians (92%) and a part of the familial PC risk group (90%). The remaining 10% were mutation carriers. Of the 278 HRIs, 54 had only one radial or linear EUS; 25 HRIs had radial EUS examination alone and 29 HRIs had linear EUS examination alone. The remaining 224 (80.6%) HRI had tandem radial and linear EUS in a randomized order (Figure 1).

Using a per-patient analysis, the prevalence of > 1 pancreatic lesions was 43.2% (120/278). In these 120 HRIs with pancreatic lesions, defined as a cystic lesion, a solid lesion, or a dilated main pancreatic duct, the majority had cystic lesions (99/120; 82.5%) with mean size of 0.8cm (range 0.1-8cm). In HRIs who underwent a single EUS exam, linear EUS detected more pancreatic lesions than radial EUS (65.4% vs 39.5%, p=0.01). In those who had 2 EUS examinations in tandem, 16 of the 224 (7.1%) HRIs had lesions missed during the initial EUS. Of these 16 HRIs with missed lesions, 11 (9.8%) had radial followed by linear (radial/linear) EUS and 5 (4.5%) had linear followed by radial

(linear/radial) EUS (p=0.03). The missed lesions were distributed throughout the pancreas (p=N.S.). The average size of the lesions missed by radial EUS was small (mean 0.57cm, range 0.3-1.2cm) and similar (p=0.63) to those missed by linear EUS (mean 0.47cm, range 0.3-0.6cm).

In the 224 HRIs who underwent tandem EUS examinations, there were 229 pancreatic lesions identified in total. In a per-lesion analysis, 109 lesions were detected by any EUS in the radial/linear group. The first radial EUS yielded 73/109 lesions (67%) and the second linear EUS yielded additional 36 lesions. In the linear/radial group, a total of 120 lesions were detected. The first linear EUS detected 99/120 lesions (82.5%) and the second radial EUS yielded additional 21 lesions (Figure 2). Hence, the incremental detection rate for a pancreatic lesion during a second exam with the linear EUS was significantly higher than that for the radial EUS (33% vs 17.5%, p= 0.007).

When all of the radial EUS examinations are combined, regardless of whether it was performed as a single procedure, or as a first case or second case of a tandem procedure, radial EUS yielded 173 of 262 (66.0%) total pancreatic lesions. For linear EUS examinations, linear EUS yielded 232 of 287 (80.8%) total pancreatic lesions (p=0.0001).

Discussion:

Endoscopic endosonography (EUS) is a powerful imaging technique in the evaluation of pancreatobiliary disease and lesions. With 2 distinct designs in echoendoscope, radial array and linear array echoendoscope,[9, 10] the choice of which to use for pancreatobiliary imaging have been left to the discretion and preference of the individual endosonographer. Over the past 10-15 years, there has been a shift in increased use of linear EUS for pancreatic imaging, even in those cases where fine needle aspiration (FNA) is not indicated.[11, 12] Anecdotally, some endosonographers feel that linear EUS provides superior quality and more complete imaging of the pancreas.[19] To the best of our knowledge, no prior studies have shown the postulated superior ability of the linear EUS to image the pancreas.

This study validates the current trend in increasing preference for the curvilinear array echoendoscope for pancreatic imaging. We have shown that linear array endosonography detected more pancreatic lesions when compared to radial array endosonography in a cohort of asymptomatic high-risk individuals (HRIs) undergoing screening program through the multi-center American Cancer of the Pancreas Screening (CAPS 3) Consortium study.

Furthermore, akin to the field of screening colonoscopy and adenoma detection, there is a “second-pass effect” with additional pancreatic lesions detected with the second EUS exam in a tandem study for both the radial and linear EUS. However, this effect was significantly greater when the linear EUS was used as the second imaging modality

after the initial radial EUS examination. When the initial screening examination was performed with radial array echoendoscope, 1/3 of the pancreatic lesions were missed; when the initial screening examination was performed with linear EUS, less than 20% of the pancreatic lesions were missed. Reassuringly, all of missed lesions were relatively small in size and well-distributed throughout the pancreas. Overall, the per-patient miss rate for pancreatic lesions in HRI was significantly lower for linear EUS compared to radial EUS.

There are several strengths of the current study. It is an embedded study within a large, multi-center study involving 5 centers using site-specific EUS equipments, which speaks to its generalizability across multiple academic facilities. Second, the study was randomized in the order of EUS examination in the tandem study, which each patient serving as his/her own control in terms of participant variables. Third, the cohort involved asymptomatic HRIs enrolled in a screening program, which consequently required complete imaging of the pancreas in a standardized fashion, instead of focusing on a target lesion.

Our study also had several limitations. All of the EUS examinations were performed only by expert endosonographers. Furthermore, majority of the pancreatic lesions detected in the study could not be verified by pathology since most were not surgically resected.

In conclusion, the results of our study suggest that linear EUS should be the echoendoscope of choice when imaging the pancreas, even when biopsy or therapeutic interventions is not expected.

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Table 1: Baseline High-Risk Individuals (HRI) Characteristics

	Total (N=278)
Risk Group	
- Familial PC	250 (90%)
- BRCA2 + PC relative	25 (9%)
- Peutz-Jeghers syndrome	3 (1%)
Race	
- Caucasian	256 (92.1%)
- African American	3 (1.1%)
- Native American	2 (0.7%)
- Asian/Pacific Islander	1 (0.4%)
- Other	16 (5.8%)
Gender	
- Male	143 (51.4%)
Age	
Mean: 56.1 years (range 25.6-86.5)	
- $\geq$ 50 years	197 (70.9%)

Figure 1: Study Schema

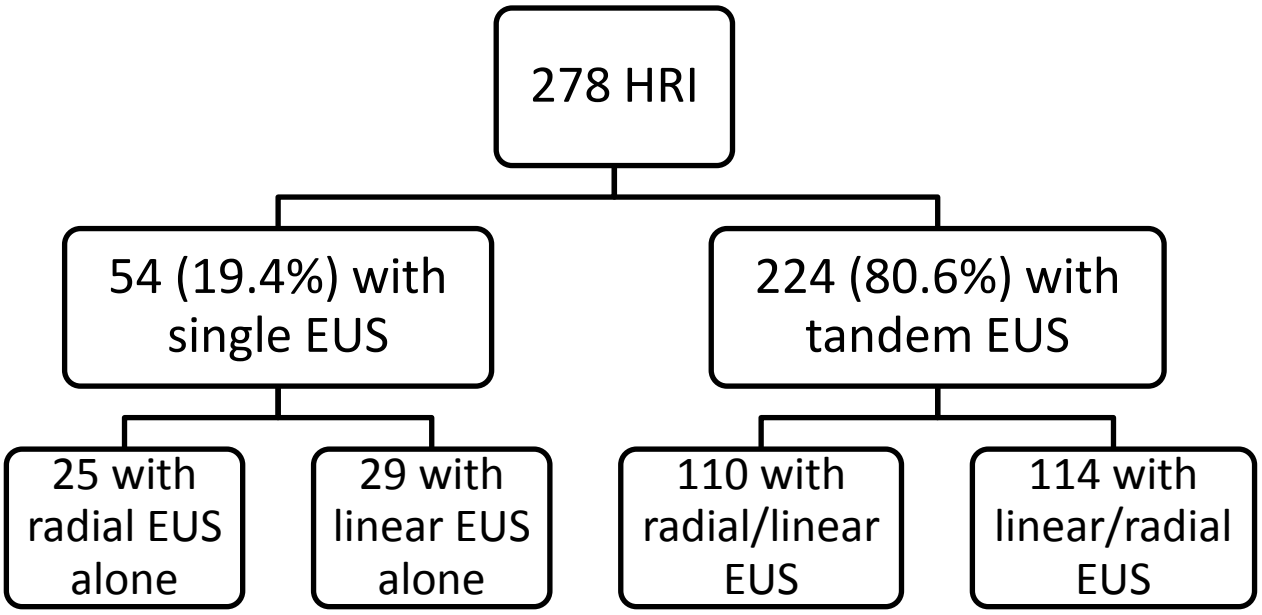
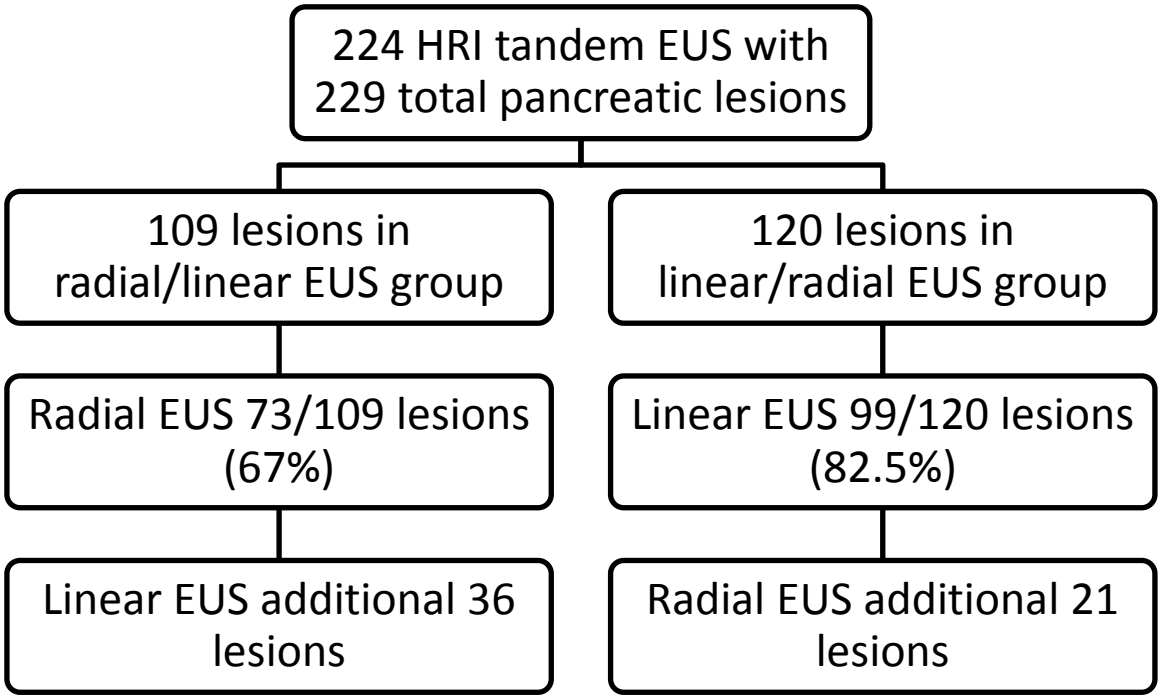


Figure 2: Yield of HRI who underwent tandem EUS examinations



Chapter 3: Novel filter clot cellblock technique is associated with increased sample adequacy and diagnostic accuracy rate of EUS-FNA

Introduction

Endoscopic ultrasound with fine needle aspiration (EUS-FNA) has become a powerful tool in diagnosing and obtaining pre-operative cytologic samples of enlarged lymph nodes and lesions in the upper gastrointestinal tract, pancreas, and rectum. Overall diagnostic rates of EUS-FNA range from 52% to 92% depending upon the site of tissue acquisition.[1-16] Diagnostic rates also vary according to procedure indication and type of lesion. EUS-FNA has sensitivity of approximately 75-90% and specificity of nearly 100% for the diagnosis of a malignant-appearing pancreatic mass lesions.[1, 4-6, 8, 11, 12, 16] EUS-FNA has sensitivity of 72-100%, specificity of 93-100%, and accuracy rate of 86-100% for the evaluation of lymphadenopathy.[2, 3, 7, 9, 11-16] However, for gastrointestinal submucosal lesions, EUS-FNA has a lower sensitivity of 50-60%, specificity of 25-100%, and accuracy rate of 38-81%.[11-13, 16]

The variability of the diagnostic rates of EUS-FNA may, in part, be due to the limited cellularity and loss of the architectural features in the EUS-FNA samples. One potential method of improving the diagnostic yield of EUS-FNA is the filter clot cellblock technique.

The filter clot (FC) technique has been used by Japanese pulmonologists for processing ultrasound-guided transbronchial needle aspirate (TBNA) samples [17]. This technique was introduced to our institution in 2009 for pulmonary indications but has yet been studied

for potential use in gastrointestinal indications. Preliminary analysis of the diagnostic yield of TBNA for mediastinal lesions at our institution showed a significant improvement in the cellularity of the samples processed using the filter cellblock technique, allowing for immunohistochemical staining for diagnostic and prognostic tumor markers (personal communication). The aim of our study was to compare the diagnostic yield and accuracy of the filter clot (FC) cellblock technique to the standard (STN) cytological cellblock technique of processing EUS-FNA samples.

Patients and Methods

Study Design, Setting, Participants

This single center non-randomized retrospective concurrent cohort study was conducted in a tertiary care academic medical center. Using data from our cytopathology and EUS databases, a retrospective review was performed for consecutive patients who underwent EUS-FNA with on-site cytopathologic evaluation at The Johns Hopkins Hospital from January 2009 to February 2010 for: (1) pancreatic solid or solid/cystic mass lesions, (2) mediastinal or intra-abdominal lymph nodes or soft tissue masses, and (3) gastrointestinal submucosal masses. Patients were excluded if the pancreatic lesion was entirely cystic with no solid component. All patients gave written informed consent for standard of care EUS-FNA. The study was approved by the Johns Hopkins Institutional Review Board for Human Research.

All pertinent patient demographics, endoscopic (indication for FNA, lesion size) data, surgical pathology, cytopathology, and long-term clinical follow-up were abstracted from electronic patient records.

EUS-FNA

All EUS-FNA examinations were performed by one of 3 experienced endosonographers with intravenous propofol sedation and monitored anesthesia care using linear echoendoscopes. All procedures had routine on-site cytological evaluation by experienced cytotechnologists for preliminary sample processing and preliminary assessment of sample adequacy to help guide specimen acquisition. Standard technique for EUS-FNA sampling was used. A 22-, 25-, or 19- gauge FNA needle (Cook Medical, Bloomington, IN) was inserted into the lesion and 10 cc of suction applied with "to-and-fro" movement of the needle tip. The choice of needle size and the total number of needle passes were determined by the endoscopist based upon the target lesion type, location, and size. If sampling with the initial FNA needle did not lead to adequate cytological material, another FNA needle of either higher or lower gauge was used at the discretion of the endoscopist. Typically, a minimum of 4 needle aspirates were obtained for lymph nodes and 6 FNA passes were obtained for pancreatic masses, unless adequate material was present in prior passes.

EUS-FNA Cell Block Techniques

The EUS-FNA samples were processed in one of two ways: standard (STN) technique or filter clot cellblock technique. At our institution, the "standard technique" has been utilized since 1996 when EUS-FNA was introduced. It involves using an air-filled syringe attached to the FNA needle to express a few drops of each sample onto glass slides for direct smears. One set of direct smears was air-dried and stained with the Diff-Quik stain for on-site evaluation; another set was immediately fixed in 95% ethanol and subsequently stained with the Papanicolaou stain. The FNA needle is then rinsed with Hank's balanced salt solution (~1-2cc) and the material is collected in a sterile conical centrifuge tube. This process is repeated after each FNA attempt. The steps are performed in the EUS endoscopy room. After all of the samples are collected in the tube, the rinse is centrifuged in the cytology laboratory to collect the pellet. The supernatant is then discarded and the pellet is scraped off and submitted for cell block. The cellblock is fixed in 10% neutral buffered formalin. The cellblock is then examined after standard processing of slides and H&E staining. The final diagnosis is made after examination of the slides with cytological FNA smears and cellblock specimen.

The filter clot (FC) cellblock technique was initiated at our institution in January 2009. It involves using the stylet passed through the FNA needle to express a few drops of the sample onto the glass slide for bedside smears. A 1" x 1" piece of filter paper is placed on top of a second glass slide. The remaining sample is pushed out from the FNA needle with the stylet onto the filter paper, allowing the needle tip to build up a cone-shaped mound of tissue and blood coagulum. This process is repeated after each FNA attempt.

The tissue coagulum collected on the filter paper at the end of the procedure (Figure 1) is entirely submitted for cellblock and is fixed in 10% neutral buffered formalin. The formalin-fixed clot is then processed using standard techniques for preparation of stained slides for pathologic examination. The final diagnosis is made after examination of the slides with cytological FNA smears and cellblock specimen.

The primary endpoints of the study were: (1) sample adequacy, which was defined as the percentage of STN and FC samples with material adequate for diagnosis and (2) diagnostic accuracy, which was defined as the percentage of accurate diagnosis attained on FNA samples in patients with surgical pathology and/or long-term clinical follow-up as the reference standard. Secondary endpoints were sample adequacy and diagnostic accuracy by type of lesion (EUS-FNA indication).

Statistical Analysis

The performance characteristics of the FNA sample processing techniques were calculated for all patients and by indication using the final diagnosis based upon surgical pathology or the results of long-term clinical follow-up after 6 months as the gold standard. For patients who did not undergo surgical resection, final diagnosis of malignancy was made if patient had cytology specimen diagnostic for malignancy with clinical progression or death from the malignancy. For patients deemed to have a benign process by EUS-FNA who did not undergo surgical resection, they were followed

clinically for at least 6 months, in accordance with other previous studies in this field. The patients needed to be without any clinical indication of malignant process, with no evidence of disease progression and/or with resolution of imaging abnormality during the clinical follow-up period (based on patient record review) for the diagnosis of benign disease.

Fisher's exact test and univariate logistic regression analysis were performed prior to multivariate logistic regression to analyze the effect of the filter cellblock technique on sample adequacy and diagnostic accuracy. All statistical analyses were performed using the Stata software, version 9.2 (Stata Corp, College Station, Texas).

Results

During the study period, 320 EUS-FNA examinations for pancreatic mass lesions, lymph nodes, soft tissue masses, or gastrointestinal submucosal masses were performed. A total of 311 patients (mean age of 62.3 +/- 12.9 years) were included in the study. One hundred fifty five were males (49.8%). Of the 320 total EUS-FNA cases, 78 samples were processed using the FC cellblock technique (24.4%) while the remaining 242 (75.6%) were processed using the standard technique.

Table 1 details the indications for EUS-FNA procedures during the study period. The most common indication for the EUS-FNA was solid pancreatic mass (n=123, 38.4%).

Pancreatic cyst with a solid component or mural nodule (n=91, 28.4%) was the second most common indication. For these lesions, the targets of the tissue sampling were the solid components or mural nodules within the cystic portions of the lesion. Other indications included lymph nodes (n=56, 17.5%), submucosal mass lesions (n=15, 4.7%), and miscellaneous indications (pancreatic and biliary stricture and adrenal mass). One hundred sixty nine (53%) of the 320 target lesions were small (< 3 cm). The size of the lesions targeted for EUS-FNA ranged from 3.5 mm to 7.4 cm (mean of 2.6 cm $\pm$ 1.4 cm).

Sample Adequacy

Table 2 shows the comparison of the STN and FC cellblock techniques for sample adequacy. Two hundred forty of the 320 EUS FNA samples (75%) were deemed to have adequate cellularity to make a cytological diagnosis by final cytological evaluation. Overall, significantly more FC samples had adequate cellularity compared to STN technique (87% versus 71%, p=0.004). When results were analyzed by EUS-FNA indication (lesion type) (Table 2), there were numerical differences between FC and STN technique adequacy rates, but these did not reach statistical significance.

Overall, the EUS-FNA samples processed with the FC cellblock technique were more likely to have a higher rate of sample adequacy when compared to the STN technique (OR 2.77, 95% CI 1.35-5.68, p=0.006). Figures 2 and 3 show representative cytological samples obtained during EUS-FNA for evaluation of solid pancreatic masses. The FC cellblock technique was also independently associated with improved sample adequacy

of EUS-FNA with an odds ratio of 2.94 (95% CI 1.26-6.90, p=0.013), even after controlling for the lesion size and the lesion type (indication for the EUS-FNA) (Table 3).

Diagnostic Accuracy

The final diagnosis was confirmed by pathologic examination of the resected specimen and/or clinical and radiology follow-up for at least 6 months in 171 of the 320 EUS-FNA procedures. In these samples, the FC cellblock technique was associated with a significantly higher overall accuracy of EUS-FNA diagnostic samples compared to the STN technique (92% versus 79%, respectively, p =0.03). The likelihood of an accurate diagnosis for all target lesions was almost 3 times greater when the FC cellblock technique was used (OR 2.96; 95% CI 1.06-8.24; p=0.04).

After stratifying by lesion type (indication for EUS-FNA), there were numerical increases in the diagnostic accuracy rate using the FC technique over the standard technique for each subgroup. The greatest potential difference in diagnostic accuracy between the FC and STN techniques was for lymph nodes (75-92%, p= 0.05). After controlling for the type of lesion, the FC cellblock technique was independently associated with improved diagnostic accuracy of EUS-FNA samples (OR 2.94, 95% CI 1.06-8.21, p = 0.04).

Discussion

Endoscopic ultrasound with fine needle aspiration (EUS-FNA) is an important tool for obtaining tissue for diagnosis of pancreatic lesions, mediastinal and intra-abdominal lymphadenopathy and soft tissue masses, and gastrointestinal submucosal lesions. With relatively low rates of complications and tumor seeding, it has increasingly become the technique of choice for pre-surgical and non-surgical tissue acquisition. However, the diagnostic yield of EUS-FNA varies by indication and can be relatively low when sampling submucosal masses and indeterminate pancreatic masses.[1-16] Hence, here is continued interest in improving techniques to increase the overall sample adequacy and diagnostic accuracy rate of EUS-FNA.

We describe the first application of the FC cellblock technique for processing EUS-FNA samples from gastrointestinal, pancreatic, and mediastinal lesions. The results of our study suggest that the FC technique of processing EUS-FNA samples is associated with increased overall sample adequacy and diagnostic accuracy rates of 87% and 92%, respectively, when compared to the STN cytological technique. Importantly, EUS-FNA specimens that were processed with the FC cellblock technique were more likely to be diagnostic and associated with an accurate final diagnosis. When the sample adequacy and accuracy of FC and STN cellblock samples were compared by lesion type, there were differences potentially favoring the FC technique but these were not statistically significant. This might be explained by the limited sample sizes for each type of lesion (EUS FNA indication), the heterogeneous patient population, variability in FNA

technique and needle type, non-prospective data collection, and other unknown confounding factors.

The FC cellblock technique may provide several advantages over the STN technique for processing EUS FNA samples. Multiple manipulations of the samples are required in the standard technique, and each step could potentially lead to some small cell loss affecting overall yield. First, an air-filled syringe is used to express the sample from the FNA needle at our institution, which may be problematic as the material could spray out in an uncontrolled manner and may adversely influence yield. Each EUS-FNA passes are collected with a rinse of Hank's balanced salt solution which dilutes the concentration of the cells in the tube, and the solution then needs to be concentrated in a centrifuge. Second, the supernatant is discarded after centrifugation and some cells may be lost during this process. Third, cells could also be lost during the actual pellet transfer, since the technician has to physically scrape the pellet from the bottom of the centrifuge tube to transfer the pellet to the cellblock. With the FC technique, the cellular material remains embedded in the blood clot and is never disassociated from the coagulum.

Another potential advantage of the FC cellblock technique is that it is technically easy, requiring no additional specialized skills for the assistants or cytology technician. No additional cytology or surgical pathology equipment is required, including a centrifuge. Although our study did not evaluate procedure time, the preparation of the tissue coagulum was accomplished quickly within a few minutes and there seemed to be no

significant increase in the overall EUS procedure time and in-room sample processing time using the filter clot cellblock technique. The FC cellblock technique may also decrease the need for on-site cytological evaluation if it can improve the overall yield of the EUS-FNA sample.

Our study has some limitations. This was a single-center, non-randomized retrospective study at a tertiary care American medical institution. Hence, we cannot control for important factors that influence the outcomes of EUS-FNA, such as number of needle passes and gauge of needle. Furthermore, there was no pathologic confirmation for the non-surgical cases, relying on long-term clinical follow-up of at least 6 months to discriminate between benign and malignant lesions. This limitation is inherent to all diagnostic tests performed on non-surgical patients that rely upon pathologic diagnosis as the reference standard. Our study did not include enough patients to demonstrate a potential difference between the STN and CB techniques for different EUS-FNA indications, but there were interesting trends that need further study. Finally, the overall negative predictive value of EUS-FNA at our institution was relatively low during the study period, possibly due to factors that influence disease prevalence, such as case mix and selective use of EUS-FNA for indeterminate masses as opposed to routine application prior to surgery. Despite its limitations, this study provides data on the potential for the FC cellblock technique as an alternative method for processing EUS-FNA samples.

In conclusion, the use of the filter clot technique for processing EUS-FNA samples was associated with an increased sample adequacy and diagnostic accuracy compared to standard cytological technique. By increasing specimen cellularity, the filter clot technique may potentially improve the feasibility for immunohistochemical staining and other studies for specific molecular markers that are important for tumor diagnosis, treatment response, and prognosis.

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Table 1: Indications for EUS-FNA

Indications for EUS-FNA	N (%)	Mean size + SD (in cm)
Solid pancreatic mass	123 (38.4%)	2.94 ± 0.14
Cystic/solid pancreatic mass	91 (28.4%)	2.29 ± 0.14
Lymph nodes	56 (17.5%)	1.91 ± 0.19
Submucosal mass	15 (4.7%)	2.57 ± 0.33
Miscellaneous indications (including pancreatic and biliary stricture on imaging, and adrenal mass).	35 (10.9)	3.28 ± 0.35
Total	320 (100%)	2.6 ± 1.4

Table 2: Percent Sample Adequacy of EUS-FNA in 320 Lesions

Indications for EUS-FNA	Standard Technique	Filter Cellblock Technique
Solid pancreatic mass	86%	90%
Cystic/solid pancreatic mass	63%	75%
Lymph nodes	70%	94% *
Submucosal mass	44%	83%
Overall	71%	87% **

* p=0.05 ** p=0.004

Table 3: Sample Adequacy of EUS-FNA in 320 Lesions

Variable	Adjusted Odds Ratio	95% Confidence Interval	p-value
Filter cellblock technique	2.94	1.26 - 6.90	0.013
Lesion size	1.17	0.94 - 1.46	0.161
Indication for EUS-FNA	0.90	0.76 - 1.07	0.249

Table 4: Diagnostic Accuracy of EUS-FNA in 171 Lesions with Final Diagnosis

Indications for EUS-FNA	Standard Technique	Filter Cellblock Technique
Solid pancreatic mass	81%	94%
Cystic/solid pancreatic mass	73%	88%
Lymph nodes	75%	92% *
Submucosal mass	60%	75%
Overall	79%	92% **

* p=0.05 ** p=0.03

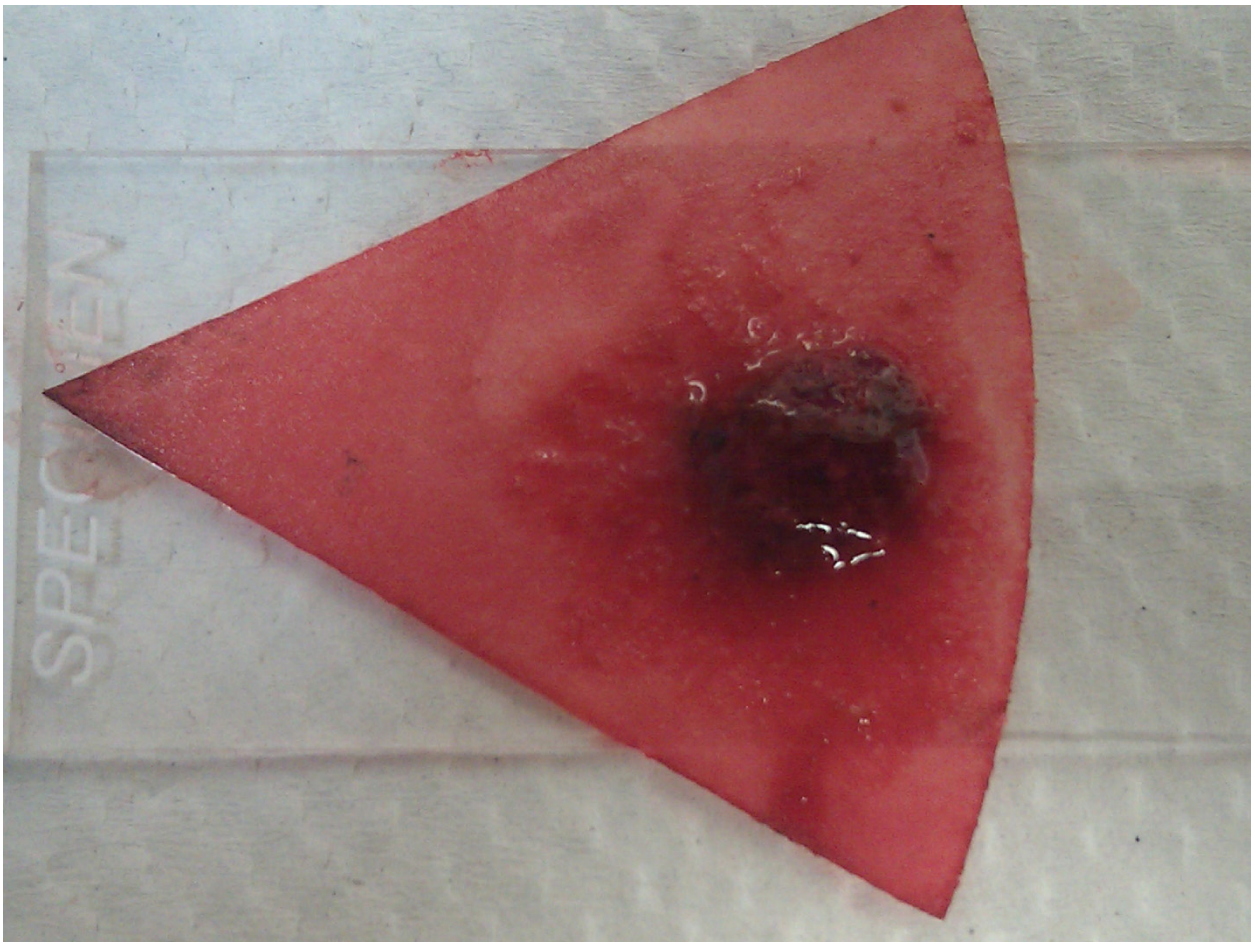

Figure 1. Close-up view of the tissue coagulum on filter paper

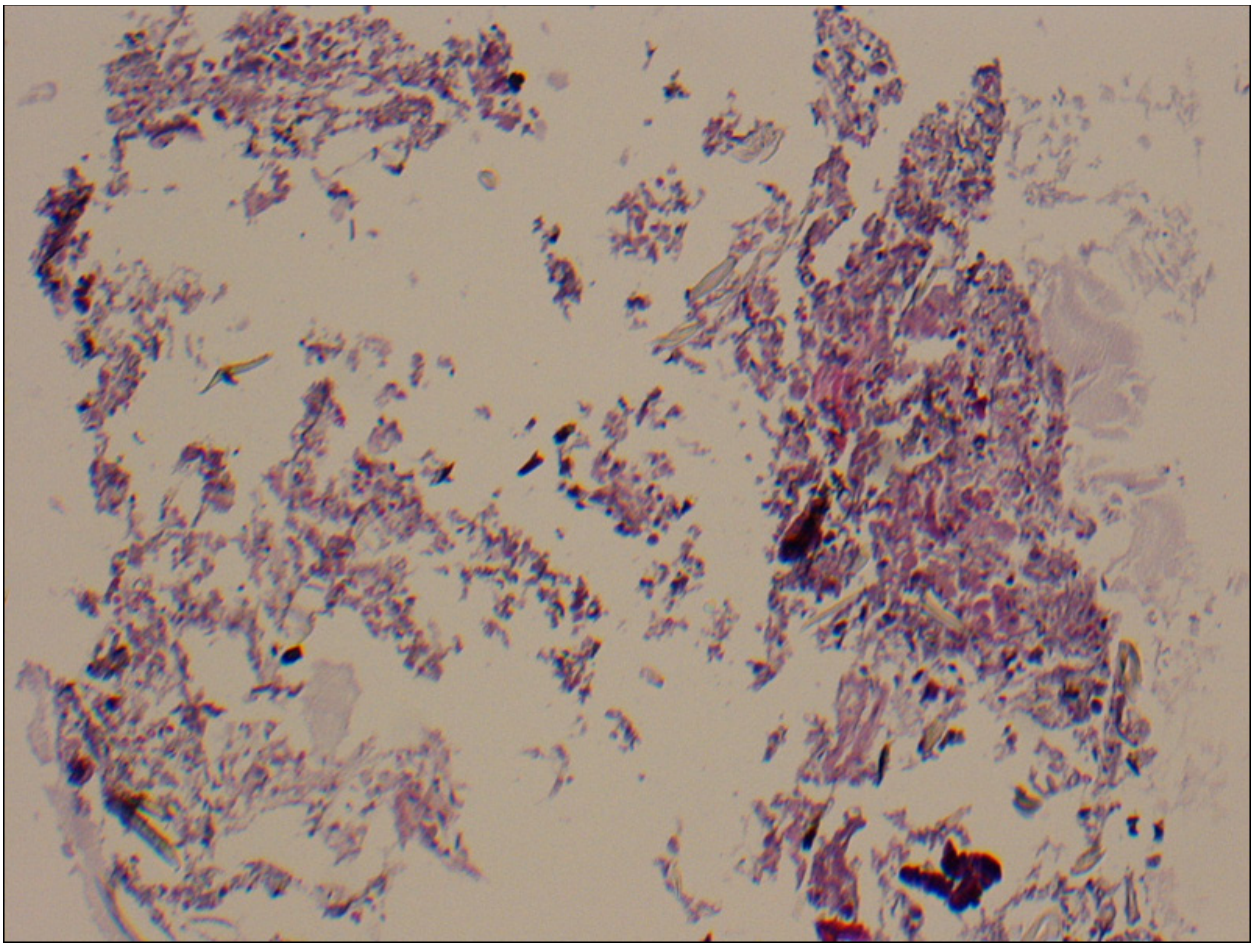

Figure 2A. Representative cellblock using the standard (STN) technique (10x) of pancreatic mass lesion with diagnosis of adenocarcinoma

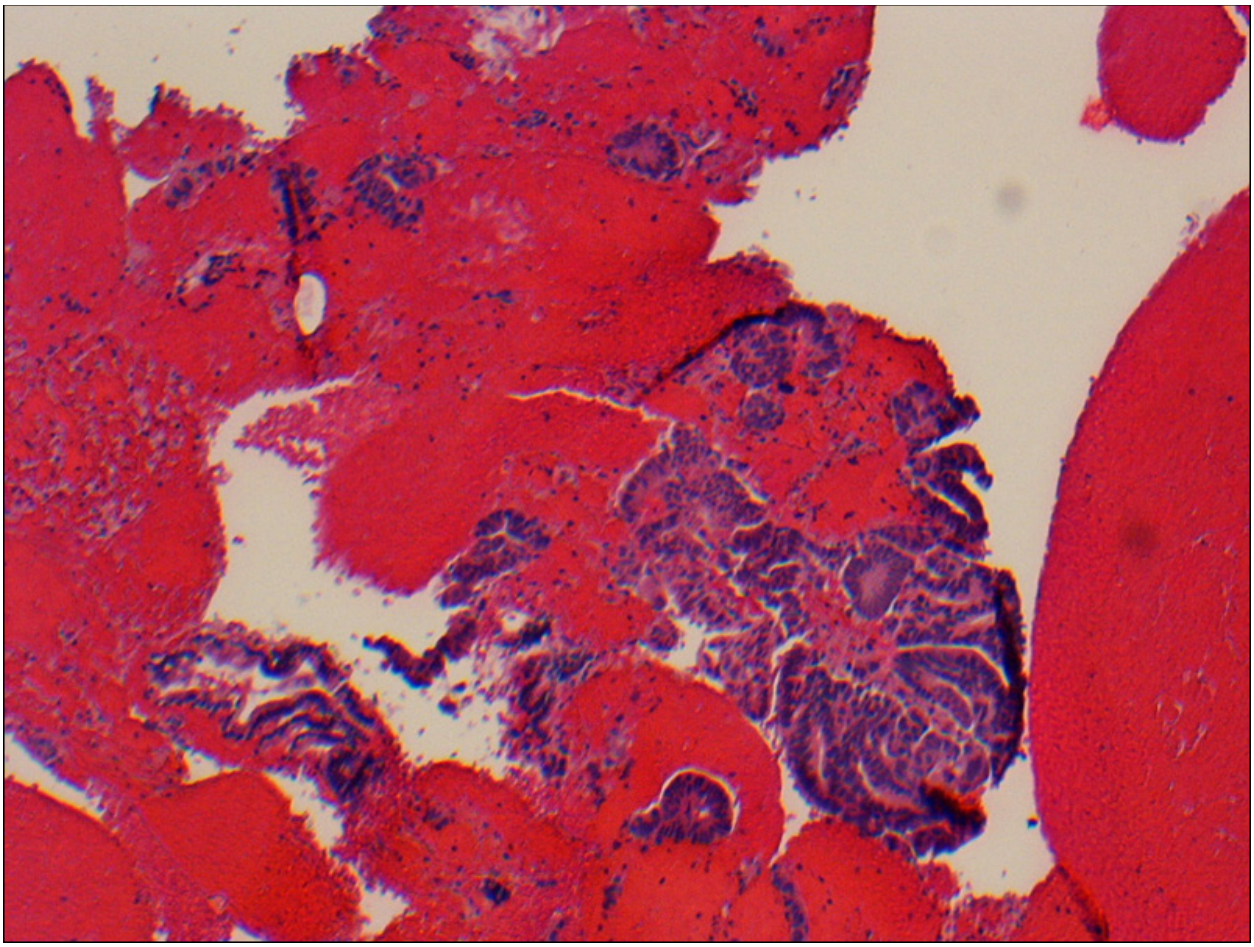

Figure 2B. Representative cellblock using the filter clot (FC) cellblock technique (10x) of pancreatic mass lesion with diagnosis of adenocarcinoma

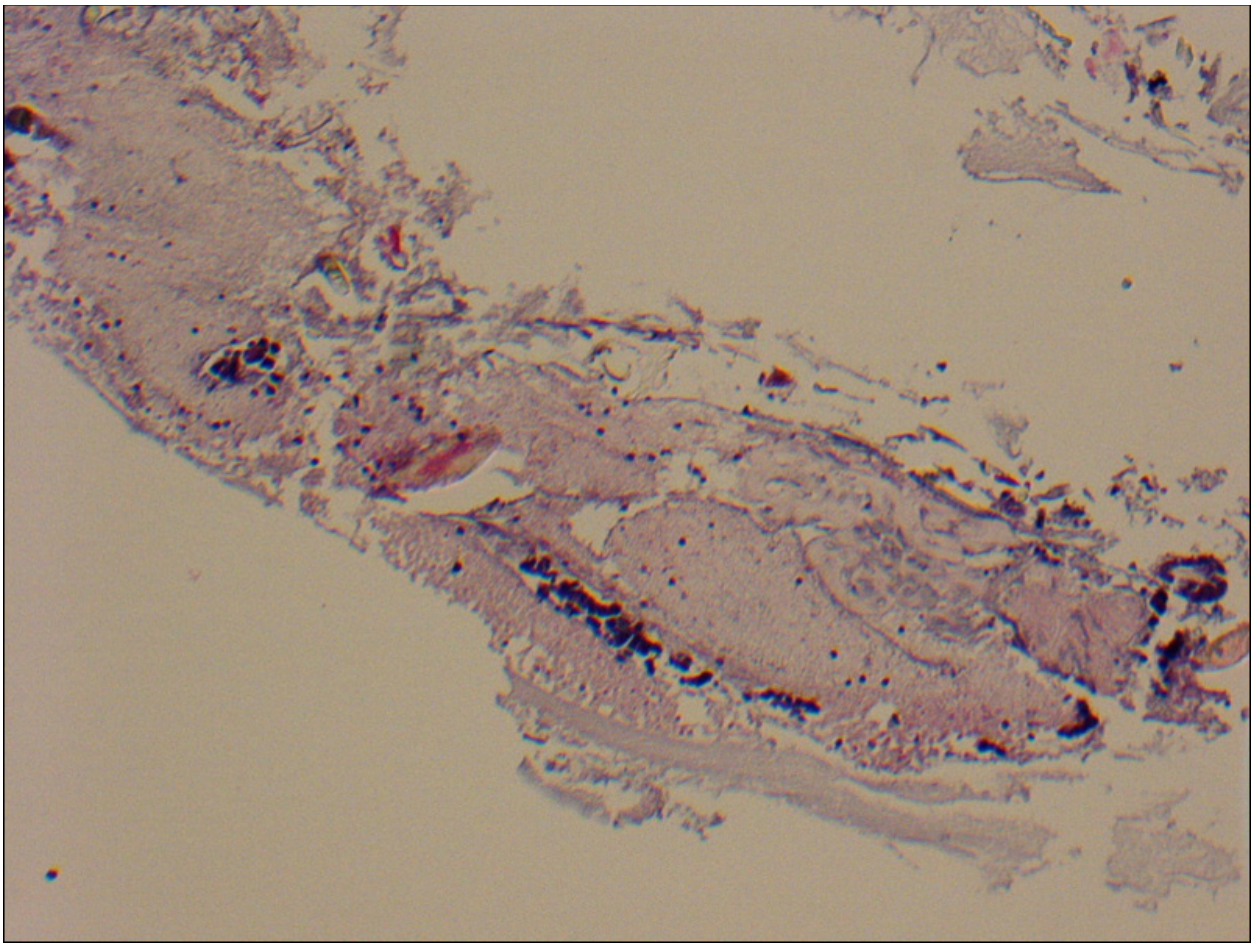

Figure 3A. Representative cellblock using the standard (STN) technique (10x) of pancreatic mass lesion with diagnosis of pancreatic neuroendocrine tumor

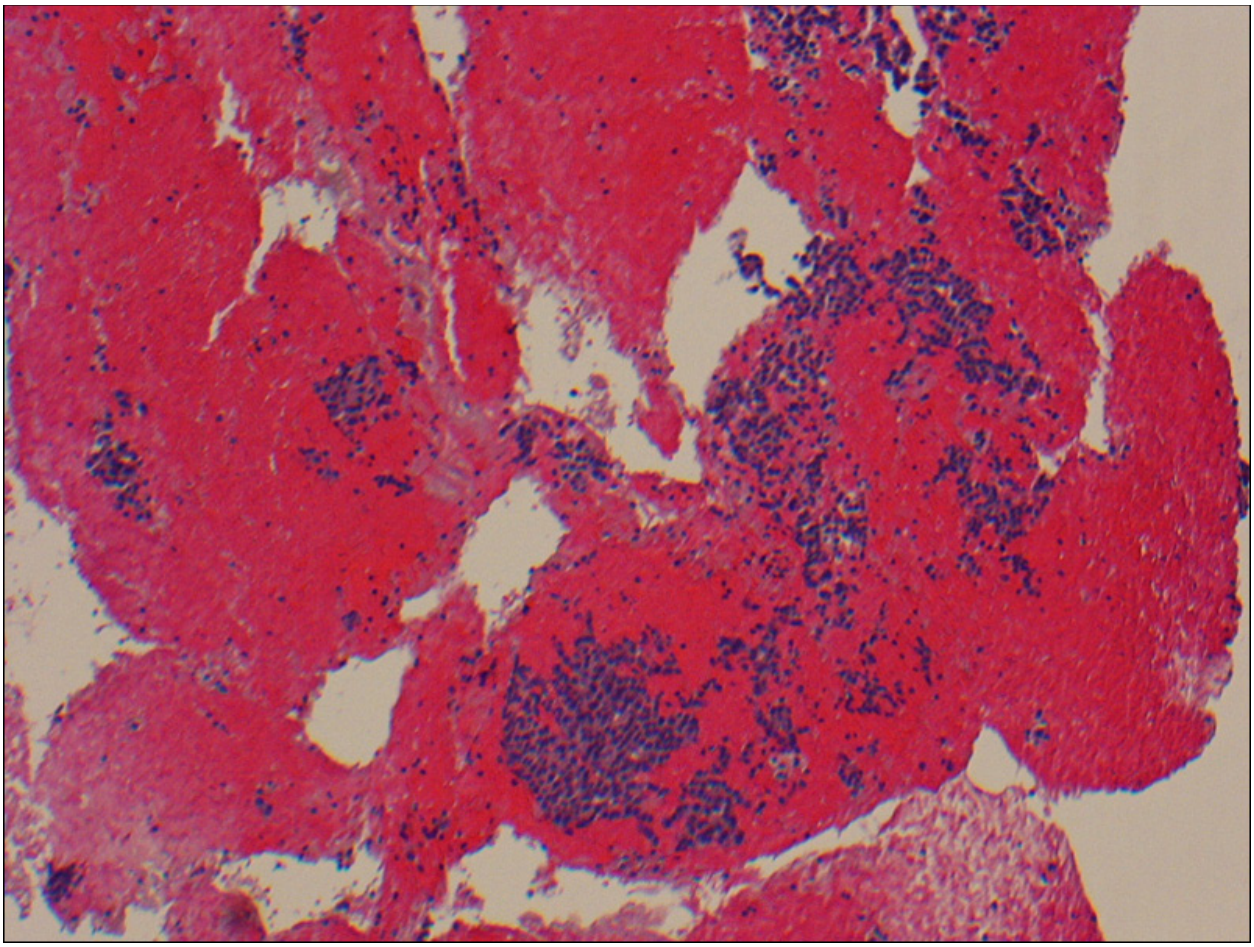

Figure 3B. Representative cellblock using the filter clot (FC) cellblock technique (10x) of pancreatic mass lesion with diagnosis of pancreatic neuroendocrine tumor

Chapter 4: DPC4 status of EUS-FNA samples in patients with pancreatic adenocarcinoma is correlated with pattern of failure

Adapted with permission from: Shin EJ, Khashab M. The role of endoscopy in the treatment, management, and personalization of pancreatic cancer. Curr Probl Cancer. 2013 Sep-Oct;37(5):293-300

Introduction:

Despite significant advances in medicine over the past decade, pancreatic adenocarcinoma remains one of the deadliest diseases being the fourth leading cause of cancer-related deaths in the United States for both males and females, with an estimated 45,220 new cases and 38,460 deaths in 2013.[1] In stark contrast to the death rates for other leading causes of cancer death (lung, colorectal, breast, and prostate), which have declined since 2003, the mortality rate from pancreatic adenocarcinoma has increased during the same time period. Therefore, early detection, diagnosis, and accurate stratification of patients with pancreatic mass lesions are paramount in providing timely, optimal care. As such, endoscopic interventions are emerging as increasingly important diagnostic and therapeutic modalities in the management of patients with pancreatic cancer.

Personalization of Pancreatic Cancer Therapy

There has been a growing interest in personalized medicine, especially in the field of cancer treatment. It is now routine practice in lung, colorectal, breast, and prostate cancer to utilize the biomarker status of the primary tumor to tailor oncologic therapies to the individual patient. Prognostic biomarkers "provide insight into the natural history of disease, including survival and recurrence pattern," while predictive biomarkers "predict response to treatment."[2, 3] Molecular profiling of tumors to improve treatment and outcome is an emerging concept in pancreatic cancer. Multiple prognostic

biomarkers (MUC-1, MSLN, SMAD4/DPC4, FOSB, KLF6, NFKB12, ATP4A, GSG1, and SIGLEC11) and predictive biomarkers (HuR, HENT1, RRMI, ERCC1, and SPARC) have been investigated in pancreatic adenocarcinoma, with promising initial results.

DPC4 (a.k.a. SMAD4) is a tumor suppressor gene inactivated in ~55% of pancreatic ductal adenocarcinomas[4] and its protein product functions in the TGF beta/Smad signaling pathway. Dpc4 immunolabeling has been shown to be an accurate predictor of DPC4 gene status.[5] Previous studies have shown an intriguing association between DPC4 gene mutation status of primary pancreatic cancers and patient outcome.[6-9] In an autopsy series, *DPC4* gene status of the primary tumor appears to correlate with pattern of recurrence and disease failure in patients with pancreatic adenocarcinoma.[8] Those with *DPC4* inactivation, by either deletion or mutation of the gene, correlated with a widespread metastatic disease phenotype, while those with an intact DPC4 status correlated with a locally advanced/oligometastatic disease phenotype.[6-9] The ability to identify the disease phenotype may have an impact on tailoring clinical management. For example, patients with loss of DPC4 expression may benefit from systemic rather than locoregional therapy given the higher risk of widely metastatic disease recurrence, while patients with intact DPC4 status may benefit from locoregional control with adjuvant chemoradiation and cytoreductive treatment.[10, 11] Therefore, it will become increasingly more important to obtain diagnostic samples adequate for biomarkers studies in patients with pancreatic mass lesions.

Role of Endoscopic Ultrasound (EUS) in Diagnosis and Obtaining Samples for Biomarker Studies

There are several methods of obtaining diagnostic samples in patients with pancreatic masses: endoscopic ultrasound-fine needle aspiration (EUS-FNA), percutaneous image-guided tissue sampling by computed tomography (CT) or ultrasound (US), and surgical biopsy. In patients with clearly resectable lesions on cross-sectional imaging, there still remains a debate whether a pre-operative biopsy is needed before undergoing definitive surgical resection, since a negative biopsy result in this setting would most likely not alter the plan for surgery.[12, 13] Nonetheless, EUS changes management in about 5% of these patients with other etiologies for pancreatic masses (*e.g.*, autoimmune pancreatitis, metastatic disease, and lymphoma). For all other patients with a pancreatic mass, biopsy sampling for definitive diagnosis is a critical first step in guiding clinical management. EUS-FNA is becoming the preferred sampling technique in this population, as it is safe with diagnostic accuracy ranging between 62% and 96%, which can be improved with availability of on-site cytopathology to evaluate sample adequacy.[13-17] EUS-FNA appears to be superior to image-guided (CT or US) biopsy in terms of accuracy of diagnosis of pancreatic cancer[18] as well as safety since it carries a significantly lower risk of peritoneal seeding.[19] There is also some evidence that EUS-FNA of a pancreatic mass is more cost-effective when compared to CT-guided biopsy and surgery.[20]

A major disadvantage of the standard EUS-FNA is that it often yields small cytologic samples without conservation of the tissue architecture, which can limit its use for immunohistochemical (IHC) and potential biomarker studies.[21] Currently, adequate specimens of pancreatic tissue for molecular marker staining are often only obtained from surgical resection specimens. Therefore, for patients deemed unresectable or for those patients who are unable to undergo surgery, Dpc4 immunolabeling of the tumor may be either impossible or only possible at autopsy. However, there has been increasing interest in improving cellular yield of FNA samples for biomarker studies with new samples acquisition techniques, including filter clot (FC) cellblock technique, and new biopsy devices.

The purpose of this study is to determine whether DPC4 gene status using EUS-FNA samples correlate with clinical outcome, which may help with pre-operative and pre-treatment prognostic stratification of patients. There are molecular markers which may have implications for overall prognosis of patients with pancreatic adenocarcinoma and in treatment strategy. Evaluation of molecular marker status using EUS-FNA samples will enable testing of molecular markers to predict overall prognosis and may aid in strategizing individual treatment plan in patients in whom surgical resection is not curative.

Methods:

Study Design and Study Population:

This retrospective study was conducted in a single tertiary academic medical center. Using data from our cytopathology and clinical databases, a review was performed for patients who underwent EUS-FNA with a final diagnosis of pancreatic adenocarcinoma and were clinically followed at The Johns Hopkins Hospital (JHH). Patients were excluded if patient did not have a EUS-FNA performed at JHH or did not have clinical follow-up with radiologic imaging examination (computed tomography (CT) and/or magnetic resonance imaging (MRI)) at JHH. All patients gave written informed consent for the standard of care clinical EUS-FNA. The study was approved by the Johns Hopkins Institutional Review Board for Human Research.

All pertinent patient demographics, tumor size and pathology, DPC4 status, and clinical follow-up were abstracted from electronic patient records and/or cytopathology database.

DPC4 Status Determination:

Archival FNA material was obtained for confirmation of diagnosis of adenocarcinoma and evaluation of cellularity of the cell block (CB) preparation by an expert cytopathologist. Only CB preparations with adequate tumor material were submitted for immunohistochemical (IHC) staining. After IHC staining, the DPC4 status of each sample was agreed upon by 2 expert pathologists and graded as positive (intact/wild-type) or negative (lost/mutated). Figure 1 show a representative EUS-FNA sample

immunostained for DPC4 and graded as positive; Figure 2 show a representative EUS-FNA sample graded as DPC4 negative.

Statistical Analysis:

The chi-square, Fisher exact test, t test, and Wilcoxon rank-sum test were performed for categoric and numeric variables, where appropriate, to compare characteristics. Two-tailed P values less than 0.05 were considered statistically significant. All statistical analyses were performed using the Stata software package, version 11 (Stata Corp, College Station, Texas).

Results:

Patient Demographics:

A total of 72 patient EUS-FNA samples were included in the study. Forty-two of the patients were males (58.3%) with mean age of 63.9 +/- 11.0 years (range 43-84 years) and 45.8% were over the age of 65. Majority of the patients were either Caucasian (70.8%) or African-American (22.2%). DPC4 status was graded as positive in 32 and negative in 40 of the samples. With regards to baseline patient demographics, there were no statistically different variables between the DPC4 positive and DPC4 negative groups (Table 1).

Clinical Characteristics:

In this cohort, the primary tumor was located predominantly in the head or uncinate process of the pancreas (76.4%), followed by the neck or body of the pancreas (15.3%) and the tail of the pancreas (8.3%). Mean tumor size was 3.62 +/- 1.35 cm (range 1.77-10.6), with 75% of patients having tumor size ≥3cm. Only 21 (29.2%) of the patients included in this study were able to undergo surgical resection during their clinical course. All patients included in the study had locally advanced, unresectable, or metastatic disease, as potentially surgically resectable patients typically underwent primary surgical intervention without routine pre-operative biopsy at our institution. Again, with respect to the clinical characteristics of the study population, there were no statistically significant differences between the DPC4 positive and DPC4 negative groups (Table 2).

Patterns of Failure and Overall Survival:

In total, 42 patients (58.3%) had evidence of metastatic disease during their clinical course. Of those, 30 patients had DPC4 negative status in their tumor while 12 had DPC4 positive status (p=0.0013) (Table 3). Univariate logistic regression analysis revealed that DPC4 positive status was associated with lower risk of having metastatic disease as the pattern of failure (OR 0.2, 95% CI 0.07-0.55, p=0.002). Furthermore, DPC4 positive status was independently associated with decreased risk of metastatic disease progression (OR 0.19, 95% CI 0.06-0.60, p=0.004), even after controlling for age, gender, race, size of tumor, and location of tumor (Table 4). Despite the differences in the pattern of failure, there was no statistically significant difference seen in the median

overall survival between the DPC4 positive and the DPC4 negative groups (12.6 vs 12.7 months, p=0.95).

Discussion:

Pancreatic adenocarcinoma is associated with abysmal outcomes, with overall 5-year survival rate of only 6%. Traditionally, it was believed that patients with pancreatic cancer eventually died of progressive, widespread metastatic disease burden. An autopsy study from our institution refuted this claim by showing that approximately 1/8 of the patients died with zero evidence of metastasis.[8] Furthermore, they were able to show that the biomarker status of the primary tumor highly correlated with the pattern of disease failure in the study population. DPC4 gene is a mediator of canonical TGF-beta signaling pathway. The loss of Dpc4 immunolabeling in the tumor appears to be associated with an increased risk of the patient developing widespread metastasis[8] and poorer outcome following surgical resection.[6, 22] One of the important potential clinical implications of this observed association of the DPC4 gene status and the differential pattern of failure in pancreatic adenocarcinoma is that patients with DPC4 expressing cancers may derive greater benefit from targeted localized therapy while patients with DPC4 non-expressing cancers may benefit more from systemic chemotherapy.[8] However, in order for biomarker studies to be useful in a clinical setting, it is crucial to obtain appropriate samples at the time of diagnosis. Most studies published in the literature thus far evaluating biomarker studies in pancreatic cancer have used surgical

pathology specimens as pre-operative endoscopic ultrasound-guided fine needle aspirates (EUS-FNA) of the pancreas typically did not provide sufficient tissue for molecular marker analysis. Therefore, for patients with unresectable pancreatic tumors or for those who were not surgical candidates, immunolabeling of the primary tumor was not routinely feasible. However, recently, advances in new techniques and needle devices have improved the ability of EUS-FNA samples to be used for biomarker studies.

The primary goal of the current study was to determine whether DPC4 status can predict the pattern of failure in patients with pancreatic adenocarcinoma using samples obtained from EUS-FNA. To the best of our knowledge, this is the largest study to specifically address the feasibility and utility of using EUS-FNA samples to determine DPC4 status and its influence on the pattern of failure in patients with pancreatic adenocarcinoma. We have shown that the DPC4 status of the primary tumor did appear to highly correlate with pattern of failure, with patients with DPC4 positive tumors significantly less likely to develop metastatic disease phenotype when compared to those with DPC4 negative tumors. This association with decreased risk remained significant, even after controlling for multiple patient and tumor characteristics. The results of this study appear to validate the clinical significance of Dpc4 immunolabeling status in diagnostic cytology specimens of pancreatic cancer patients as well as support the results from the seminal rapid autopsy study.[8] Despite the differences in the pattern of disease progression, the DPC4 status did not appear to influence the overall

median survival duration, which, while thought-provoking, is consistent with another recently published study.[23]

The main limitation of this study is the single-center retrospective nature of the study, which carries with it the inherent risk of biases which cannot be fully controlled for, even with multivariate analyses. Furthermore, we could not standardize the clinical treatment course of the individual patients.

Despite its limitations, this study provides important data that will be useful in planning future clinical studies evaluating the potential role of DPC4 status of the primary tumor at diagnosis in tailoring surgical and oncologic treatments in patients with pancreatic cancer. In addition, the demonstration of feasibility of using EUS-FNA samples for marker analysis can provide the basis for similar studies using other markers such as KRAS mutation status, p53 immunolabeling and other markers that could be useful in predicting outcome or response to therapy.

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Table 1: Baseline Patient Characteristics

	All N=72 (%)	DPC4 NEG N=40 (%)	DPC4 POS N=32 (%)	p-value
Age (mean ± SD) in years	63.9 ± 11.0	63.2 ± 11.5	64.9 ± 10.4	0.497
Male	42 (58.3)	23 (57.5)	19 (59.4)	0.873
Race -Caucasian -African-American -Hispanic -Other	 51 (70.8) 16 (22.2) 1 (1.4) 4 (5.6)	 27 (67.5) 10 (25.0) 1 (2.5) 2 (5.0)	 24 (75.0) 6 (18.8) 0 2 (6.2)	 0.487 0.526

Table 2: Baseline Tumor Characteristics

	All N=72 (%)	DPC4 NEG N=40 (%)	DPC4 POS N=32 (%)	p-value
Size of tumor (mean ± SD) in cm	3.62 ± 1.35	3.52 ± 1.17	3.75 ± 1.53	0.484
Location of tumor				
-Head	55 (76.4)	29 (72.5)	26 (81.2)	0.385
-Body	11 (15.3)	8 (20.0)	3 (9.4)	0.213
-Tail	6 (8.33)	3 (7.5)	3 (9.4)	0.775

Table 3: Clinical Outcomes

	All N=72 (%)	DPC4 NEG N=40 (%)	DPC4 POS N=32 (%)	p-value
Pattern of Failure: Metastasis	42 (58.33)	30 (75.0)	12 (37.5)	0.001
Median Overall Survival (months)	12.7	12.6	12.7	0.955

Table 4: Pattern of Failure – Univariate and Multivariate Analyses

	Unadjusted OR (95% Confidence Interval)	p-value	Adjusted OR (95% Confidence Interval)	p-value
Age>65	0.47 (0.18-1.22)	0.121	0.51 (0.17-1.54)	0.231
Male gender	1.125 (0.43-2.92)	0.808	1.38 (0.42-4.49)	0.592
Race	1.44 (0.73-2.82)	0.292	1.29 (0.62-2.69)	0.496
Size of tumor ≥3 cm	1.57 (0.54-4.60)	0.409	1.19 (0.32-4.47)	0.792
Location of tumor	2.83 (1.02-7.85)	0.046	2.85 (0.98-2.69)	0.053
Surgical resection	0.93 (0.33-2.61)	0.895	0.84 (0.26-2.77)	0.778
DPC4 positive status	0.20 (0.07-0.55)	0.002	0.21 (0.07-0.62)	0.005

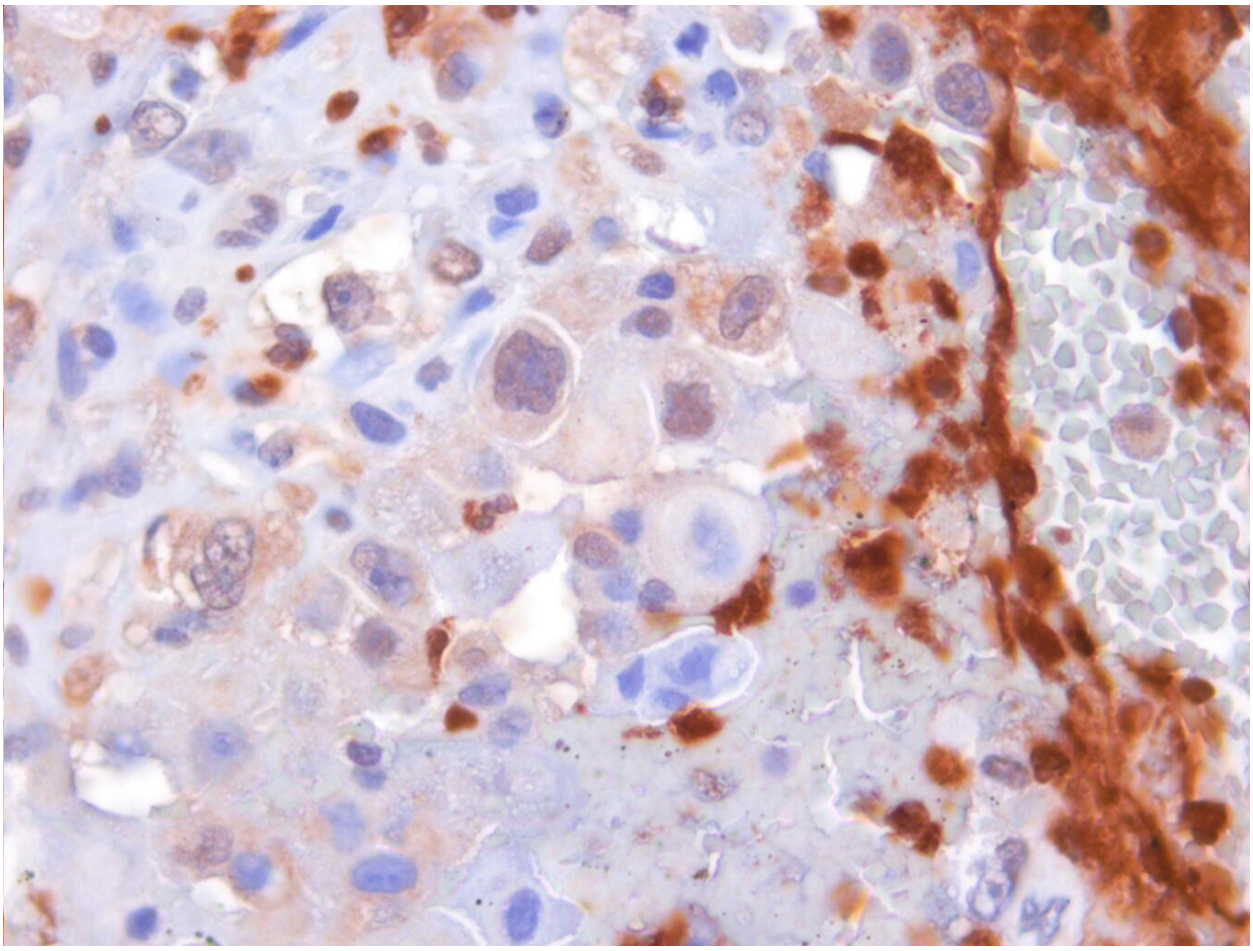

Figure 1. Representative EUS-FNA sample immunostained for DPC4 and graded as positive

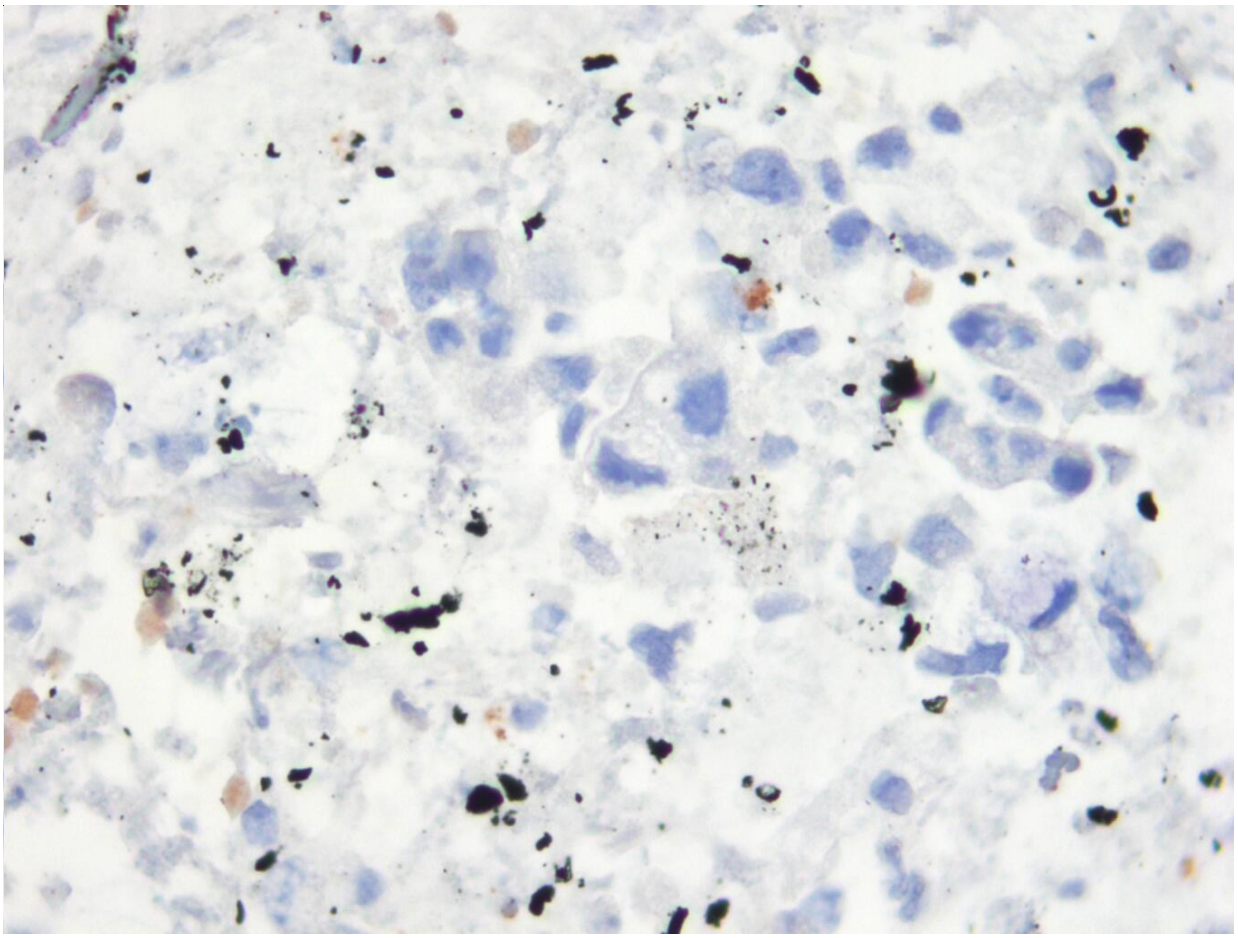

Figure 2. Representative EUS-FNA sample immunostained for DPC4 and graded as negative

Appendix 1.

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Appendix 3

CAPS 3 study Screening for Early Pancreatic Neoplasia 1

CAPS 3 EUS FORM
(to be completed by endosonographer performing EUS)

CENTER 1 = JHH 2=Mayo 3=MDACC 4=UCLA
capsno
CAPS NO: ____ ____ ____-___ - ___ ___ ___ dateEUS DATE ____ ____-___ ____-____ ____

VISIT 1 ____ baseline 2____ FU 1 year 3 ____ interim visit

ANESTH 1___versed + fentanyl 2___ GA (propofol) w/o intubation 3___GA + intubation

FORM COMPLETED BY _____, M.D., eusmd

Study quality: Excellent Good Adequate Poor

Please explain: _____

EUS EQUIPMENT eus_equip **(check all that apply)**
1 ____ Olympus 160 radial (mechanical)
2 ____ Olympus radial electronic
3 ____ Olympus CLA
4 ____ Pentax 360 deg radial
5 ____ Pentax CLA
6 ____ Other _____

ORDER OF ECHOENDOSCOPE order
1____ radial then linear
2 ____linear then radial

FINDINGS WITH FIRST ECHOENDOSCOPE
neus_1
Normal EUS 0____ No 1 _____Yes

PANCREATIC MASS OR CYST mass: "localized abnormality with echotexture"
cyst: "abnormal anechoic round or oval structure with no blood flow"

1. Number of masses _____ tot_mass
   If > 3 masses, describe the largest 3 below

2. Mass 1 eus_mass1

   a. Location of mass 1 **mllocn**

   (draw on figure and select all locations that apply)

❒head ❒uncinate ❒neck ❒body ❒tail

CAPS 3 EUS performer_2-13-07 1

b. Maximum diameter of mass: _____ cm eus_m1size

c. Solid ❒ No ❒ Yes

d. Cystic ❒ No ❒ Yes
1. Solid component ❒ No ❒ Yes
2. Nodule (focal wall or septal thickening)
a. ❒ No ❒ Yes
b. Height of nodule (in millimeters): _____ mm
3. Septations ❒ No (If no, skip to #4). ❒ Yes (if yes, go to 3a, 3b, 3c).
a. ❒Thick (>1 mm) ❒Thin (≤1 mm)
b. Number of locules or compartments
❒ one (unilocular) ❒ 2-6 ❒ > 6 ❒ 7-12 ❒ > 12
c. Size of cystic components
❒<1cm ❒1-2 cm ❒ >2 cm
4. Ductal communication ❒ No ❒ Yes

e. Margins ❒Well-defined ❒ Infiltrative or ill-defined
f. Fat ❒ No ❒ Yes

g. Echogenicity m1echog
1 ____ hypoechoic
2 ____ isoechoic
3 ____ hyperechoic
4 ____ anechoic

3. Mass 2
a. Location of mass 2 m2locn

(draw on figure and select all locations that apply)

❒head ❒uncinate ❒neck ❒body ❒tail

b. Maximum diameter of mass: _____ cm eus_m2size

c. Solid ❒ No ❒ Yes

d. Cystic ❒ No ❒ Yes
1. Solid component ❒ No ❒ Yes
2. Nodule (focal wall or septal thickening)
a. ❒ No ❒ Yes
b. Height of nodule (in millimeters): _____ mm
3. Septations ❒ No (If no, skip to #4). ❒ Yes (if yes, go to 3a, 3b, 3c).
a. ❒Thick (>1 mm) ❒Thin (≤1 mm)
b. Number of locules or compartments
❒ one (unilocular) ❒ 2-6 ❒ > 6 ❒ 7-12 ❒ > 12
c. Size of cystic components
❒<1cm ❒1-2 cm ❒ >2 cm
4. Ductal communication ❒ No ❒ Yes

e. Margins ❒Well-defined ❒ Infiltrative or ill-defined

f. Fat ❒ No ❒ Yes

g. Echogenicity **m1echog**
1 ____ hypoechoic
2 ____ isoechoic
3 ____ hyperechoic
4 ____ anechoic

4. Mass 3
a. Location of mass3 **m3locn**

(draw on figure and select all locations that apply)

❒head ❒uncinate ❒neck ❒body ❒tail

b. Maximum diameter of mass: _____ cm **eus_m3size**

c. Solid ❒ No ❒ Yes

d. Cystic ❒ No ❒ Yes
1. Solid component ❒ No ❒ Yes
2. Nodule (focal wall or septal thickening)
a. ❒ No ❒ Yes
b. Height of nodule (in millimeters): _____ mm
3. Septations ❒ No (If no, skip to #4). ❒ Yes (if yes, go to 3a, 3b, 3c).
a. ❒Thick (>1 mm) ❒Thin (≤1 mm)
b. Number of locules or compartments
❒ one (unilocular) ❒ 2-6 ❒ > 6 ❒ 7-12 ❒ > 12
c. Size of cystic components
❒<1cm ❒1-2 cm ❒ >2 cm
4. Ductal communication ❒ No ❒ Yes

e. Margins ❒Well-defined ❒ Infiltrative or ill-defined

f. Fat ❒ No ❒ Yes

g. Echogenicity **m1echog**
1 ____ hypoechoic
2 ____ isoechoic
3 ____ hyperechoic
4 ____ anechoic

PARENCHYMAL FEATURES
(bolded items are features of chronic pancreatitis used by American endosonographers)

Overall Echopattern **heterog**

1____ homogeneous
2____ mildly heterogeneous
3____ moderately heterogeneous
4____ markedly heterogeneous

echofoci
Echogenic foci 0____ No 1_____Yes
(small distinct nonshadowing hyperechoic points)

Locnfoci
Location of echogenic foci (select all locations that apply)

❒head ❒uncinate ❒neck ❒body ❒tail

estrands
Echogenic strands 0____ No 1_____Yes
(> 2 mm echogenic lines; string-like, hyperechoic structures)

locnstrn
Location of echogenic strands (select all locations that apply)
❒head ❒uncinate ❒neck ❒body ❒tail

irreg_marg
Irregular outer gland margin
0____ No 1_____Yes

Lobularity
lobularity "containing lobules – rounded homogeneous areas separated by strands of another echogenicity"

1_____Yes, focal
2_____yes, diffuse
0_____no

eus_calcif
Calcification (w/ shadowing) 0____ No 1_____Yes

eus_cyst 0____ No 1_____Yes
(abnormal anechoic round or oval structure in the pancreas without blood flow)

eus_cystnum
Number of cysts _____

eus_atrophy
Atrophy 0____ No 1_____Yes
(A-P diameter at body < 1 cm)

eus_fat
(discrete non-shadowing hyperechoic oval or round nodules larger than echogenic foci)
Fat deposition/infiltration 0____ No 1_____Yes

PANCREATIC DUCTAL CHANGES
CAPS 3 EUS performer_2-13-07 4

Eus_pdhead
MPD diam head _____ mm (measured within 2 cm from ampulla)
Eus_pdbody
MPD diam body _____ mm (measured at level of PV-SV confluence)
Eus_pdtail
MPD diam tail _____ mm (measured at level of top pole left kidney)

1. ❒ Normal pancreatic duct appearance

2. ❒ Abnormal caliber of main pancreatic duct
(normal: head > 3, body > 2, tail > 1 mm for age < 60; add 1 mm for age > 60)

a. **MPD dilation** 0____ No 1 _____Yes
(> 3 mm at head, > 2 mm in body, > 1 mm tail; measure from top of near wall interface echo to top of far wall interface echo)
❒ focal
location of focal dilation: check all that apply
❒head ❒uncinate ❒neck ❒body ❒tail

❒ diffuse

b. stricture (abnormal localized decrease in caliber) 0____ No 1 _____Yes

location of stricture: ❒head ❒uncinate ❒neck ❒body ❒tail

❒ Abrupt cutoff or obstruction

❒ **Main pancreatic duct irregularity** (coarse/uneven outline of the duct) 0____ No 1 _____Yes
mpddirreg

❒ **Echogenic MPD** wall region where the duct wall echoes are brighter than normal
echompd
0____ No 1 _____Yes

3. Main pancreatic duct wall thickening ❒ Yes ❒ No
a. ❒ smooth ❒ irregular
b. ❒ focal ❒ diffuse

4. **Stone(s) in duct** ❒ Yes ❒ No
eus_pdstone (hyperechoic lesion with acoustic shadowing within a duct)

5. **Side branches dilation** 0____ No 1 _____Yes
sdectasia (anechoic tubular structure joining or budding from main pancreatic duct)

❒ focal (select all locations that apply)
location: ❒head ❒uncinate ❒neck ❒body ❒tail
❒ diffuse

6. Saccular or cystic dilation of branch duct ❒ Yes ❒ No

a. Number _____
b. Location
❒ focal (draw on figure and select all locations that apply)

location: ❒head ❒uncinate ❒neck ❒body ❒tail

❒ diffuse

numEUSfeatures
Total number of 10 (Bolded) EUS features present: _____

euscpgrade
CHRONIC PANCREATITIS GRADE (German - Hollerback et al, Endoscopy, 2001:33(10):824-831)

0____EUS 0 - no morphologic alterations of the entire pancreas;

1 ___ EUS 1 - diffuse (or patchy) hypoechoic foci and hyperechoic septae (i. e. "pseudolobuli") located diffusely within the pancreatic parenchyma;

2____EUS 2 - EUS 1 plus gross irregularities of the main pancreatic duct (dilatation/narrowing);

3____ EUS 3 - EUS 1 and 2 plus calcifications, pseudocysts, or intraductal calculi.

COMMON BILE DUCT
Size ❒ Normal
❒ Age appropriate
❒ Prior cholecystectomy
Maximum diameter of the extrahepatic bile duct: _____ mm

PANCREATIC CANCER eus_panca
EUS findings suspicious for pancreatic cancer? 0____ No 1 _____Yes

OTHER EUS FINDINGS **othereus**
1 ____ hypoechoic ventral pancreas
2 ____ lymphadenopathy
Location of lymphadenopathy (check all that apply):
❒periportal ❒peripancreatic ❒perigastric ❒celiac ❒mediastinal

3 List other clinically significant findings requiring treatment and/or follow-up:
a. _____
b. _____
c. _____

PANCREATIC JUICE COLLECTION FROM THE DUODENUM (to be performed before FNA)
base_lav
Baseline lavage of the duodenum with 30 cc of saline followed by suction performed? 0____ No 1 _____Yes

base_vol

Baseline lavage volume collected = _____ ml

Pj_succ
PJ collection successful? 0____ No 1 _____Yes
If no, why not? _____

eus_secretin
Secretin dose administered (0.2 ug/kg): _____

eus_time
Total time PPJ collected during EUS: _____

Pj_vol
Volume 5 min sample: _____ ml

Pj_bile
PJ bile stained? 0____ No 1 _____Yes

EUS Diagnosis (before FNA) – RADIAL ECHOENDOSCOPE

1. Predicted pathology – pancreatic lesion 1
   - ❒Pancreatic adenocarcinoma – primary
   - ❒Pancreatic malignancy - metastatic
   - ❒Pancreatic neuroendocrine neoplasm
   - ❒Main duct IPMN
   - ❒Pancreatic cyst - serous cystadenoma
   - ❒Pancreatic cyst – branch duct IPMN
   - ❒Pancreatic cyst - mucinous cystadenoma/carcinoma
   - ❒Pancreatic cyst (benign epithelial cyst)
   - ❒Pancreatic cyst - pseudocyst
   - ❒Pancreatic cyst – indeterminate
   - ❒ Intrapancreatic spleen
   - ❒Chronic pancreatitis (focal)
   - ❒ Other: _____

2. Predicted pathology – pancreatic lesion 2
   - ❒Pancreatic adenocarcinoma – primary
   - ❒Pancreatic malignancy - metastatic
   - ❒Pancreatic neuroendocrine neoplasm
   - ❒Main duct IPMN
   - ❒Pancreatic cyst - serous cystadenoma
   - ❒Pancreatic cyst – branch duct IPMN
   - ❒Pancreatic cyst - mucinous cystadenoma/carcinoma
   - ❒Pancreatic cyst (benign epithelial cyst)
   - ❒Pancreatic cyst - pseudocyst
   - ❒Pancreatic cyst – indeterminate
   - ❒ Intrapancreatic spleen
   - ❒Chronic pancreatitis (focal)
   - ❒ Other: _____

3. Predicted pathology – pancreatic lesion 3
- ❒Pancreatic adenocarcinoma – primary
- ❒Pancreatic malignancy - metastatic
- ❒Pancreatic neuroendocrine neoplasm
- ❒Main duct IPMN
- ❒Pancreatic cyst - serous cystadenoma
- ❒Pancreatic cyst – branch duct IPMN
- ❒Pancreatic cyst - mucinous cystadenoma/carcinoma
- ❒Pancreatic cyst (benign epithelial cyst)
- ❒Pancreatic cyst - pseudocyst
- ❒Pancreatic cyst – indeterminate
- ❒ Intrapancreatic spleen
- ❒Chronic pancreatitis (focal)

❒ Other: _____

4. Other predicted pathology (select ALL that apply)
- ❒ Acute pancreatitis
- ❒ Chronic pancreatitis
- ❒ Fatty replacement of the pancreas
- ❒ Other: _____

EUS Diagnosis (before FNA) – LINEAR ECHOENDOSCOPE

1. Predicted pathology – pancreatic lesion 1
- ❒Pancreatic adenocarcinoma – primary
- ❒Pancreatic malignancy - metastatic
- ❒Pancreatic neuroendocrine neoplasm
- ❒Main duct IPMN
- ❒Pancreatic cyst - serous cystadenoma
- ❒Pancreatic cyst – branch duct IPMN
- ❒Pancreatic cyst - mucinous cystadenoma/carcinoma
- ❒Pancreatic cyst (benign epithelial cyst)
- ❒Pancreatic cyst - pseudocyst
- ❒Pancreatic cyst – indeterminate
- ❒ Intrapancreatic spleen
- ❒Chronic pancreatitis (focal)

❒ Other: _____

2. Predicted pathology – pancreatic lesion 2
- ❒Pancreatic adenocarcinoma – primary
- ❒Pancreatic malignancy - metastatic
- ❒Pancreatic neuroendocrine neoplasm
- ❒Main duct IPMN
- ❒Pancreatic cyst - serous cystadenoma
- ❒Pancreatic cyst – branch duct IPMN
- ❒Pancreatic cyst - mucinous cystadenoma/carcinoma
- ❒Pancreatic cyst (benign epithelial cyst)
- ❒Pancreatic cyst - pseudocyst
- ❒Pancreatic cyst – indeterminate
- ❒ Intrapancreatic spleen

❒Chronic pancreatitis (focal)

❒ Other: _____

3. Predicted pathology – pancreatic lesion 3

❒Pancreatic adenocarcinoma – primary
❒Pancreatic malignancy - metastatic
❒Pancreatic neuroendocrine neoplasm
❒Main duct IPMN
❒Pancreatic cyst - serous cystadenoma
❒Pancreatic cyst – branch duct IPMN
❒Pancreatic cyst - mucinous cystadenoma/carcinoma
❒Pancreatic cyst (benign epithelial cyst)
❒Pancreatic cyst - pseudocyst
❒Pancreatic cyst – indeterminate
❒ Intrapancreatic spleen
❒Chronic pancreatitis (focal)

❒ Other: _____

4. Other predicted pathology (select ALL that apply)

❒ Acute pancreatitis
❒ Chronic pancreatitis
❒ Fatty replacement of the pancreas
❒ Other: _____

OTHER ADDITIONAL FINDINGS WITH SECOND ECHOENDOSCOPE

_____

_____

_____

_____

EUS FNA performed 0____ No 1 ____ Yes **(If yes, go to EUS FNA form)**

Curriculum Vitae for Eun Ji Shin, MD

DEMOGRAPHIC INFORMATION

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Education and Training

1993-1997	B.A., Harvard University, Biology
1997-2001	M.D. Duke University School of Medicine
2001-2004	Internship, Johns Hopkins Hospital, Internal Medicine
2002-2004	Residency, Johns Hopkins Hospital, Internal Medicine
2004-2008	Fellowship, Johns Hopkins Hospital, Gastroenterology and Hepatology
2006-present	Ph.D. track. Johns Hopkins Bloomberg School of Public Health, Graduate Training Program in Clinical Investigation
2007-2009	Fellowship, Johns Hopkins Hospital, Advanced Therapeutic Endoscopy

Professional Experience

2008-2009	Instructor in Medicine, Johns Hopkins Hospital
2009-present	Assistant Professor of Medicine, Johns Hopkins Hospital

RESEARCH ACTIVITIES

Publications

Peer-reviewed original research articles

1. Hidalgo P, Ansari AZ, Schmidt P, Hare B, Simkovich N, Farrell S, **Shin EJ**, Ptashne M, Wagner G. "Recruitment of the transcriptional machinery through GAL11P: structure and interactions of the GAL4 dimerization domain." Genes & Development 2001;15:1007-1020.
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angiography and portal vein pressure measurements." Gastrointest Endosc. 2008 Feb;67(2):338-342.
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20. Dray X, Redding SK, **Shin EJ**, Buscaglia JM, Giday SA, Wroblewski RJ, Assumpcao L, Krishnamurty DM, Magno P, Pipitone LJ, Marohn MR, Kalloo AN, Kantsevoy SV. "Hydrogen leak test is minimally invasive and highly specific for assessment of the integrity of the luminal closure after natural orifice transluminal endoscopic surgery procedures (with video)." Gastrointest Endosc. 2009 Mar;69(3):554-60.

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22. Buscaglia JM, Dray X, **Shin EJ**, Magno P, Chmura KM, Surti VC, Dillon TE, Ducharme RW, Donatelli G, Thuluvath PJ, Giday SA, Kantsevoy SV. "A new alternative for a transjugular intrahepatic portosystemic shunt: EUS-guided creation of an intrahepatic portosystemic shunt." Gastrointest Endosc. 2009 Apr;69(4):941-947.
23. Dray X, Giday SA, Buscaglia JM, Gabrielson KL, Kantsevoy SV, Magno P, Assumpcao L, **Shin EJ**, Reddings SK, Woods KE, Marohn MR, Kalloo AN. Omentoplasty for gastrotomy closure after natural orifice transluminal endoscopic surgery procedures (with video). Gastrointest Endosc. 2009 Apr 23. [Epub ahead of print]
24. **Shin EJ**, Kantsevoy SV. "Reversed ERCP rendezvous procedure: minor papilla stenting to facilitate main pancreatic duct and common bile duct access through the major papilla." Gastrointest Endosc. 2009 Jun;69(7):e73-6.
25. Giday SA, Buscaglia JM, Althaus J, Donatelli G, Krishnamurty DM, Dray X, Ruben D, Liang D, Wroblewski R, Magno P, **Shin EJ**, Kalloo AN. "Successful diagnostic and therapeutic intrauterine fetal interventions by natural orifice transluminal endoscopic surgery (with videos)." Gastrointest Endosc. 2009 Aug;70(2):377-81. Epub 2009 Jun 11.
26. Buscaglia JM, DiMaio CJ, Pollack MJ, **Shin EJ**, Harris MD, Richards R, Chak A, Kantsevoy SV, Jagannath SB, Okolo PI 3rd. "Are large side holes associated with reduced rates of pancreatic stent occlusion? Results of a prospective study." JOP. 2009 Sep 4;10(5):496-500.
27. Lennon AM, Chandrasekhara V, **Shin EJ**, Okolo PI 3rd. "Spiral-enteroscopy-assisted enteral stent placement for palliation of malignant small-bowel obstruction (with video)." Gastrointest Endosc. 2009 Nov 5.
28. Giday SA, Dray X, Magno P, Buscaglia JM, **Shin EJ**, Surti VC, Assumpcao L, Marohn MR, Ruben D, Zhigalin A, Pipitone LJ, Kantsevoy SV. "Infection during natural orifice transluminal endoscopic surgery: a randomized, controlled study in a live porcine model." Gastrointest Endosc. 2010 Apr;71(4):812-6.
29. Dray X, Donatelli G, Krishnamurty DM, Dubcenco E, Wroblewski RJ, Assumpcao L, Giday SA, Buscaglia JM, **Shin EJ**, Magno P, Pipitone LJ, Marohn MR, Kantsevoy SV, Kalloo AN. "A 2-microm Continuous-Wave Laser System for Safe and High-Precision Dissection During NOTES Procedures." Dig Dis Sci. 2010 Apr 16.
30. Khashab MA, Lennon AM, Dunbar KB, Singh VK, Chandrasekhara V, Giday S, Canto MI, Buscaglia JM, Kapoor S, **Shin EJ**, Kalloo AN, Okolo PI 3rd. "A comparative evaluation of single-balloon enteroscopy and spiral enteroscopy for patients with mid-gut disorders." Gastrointest Endosc. 2010 Jul 7.
31. Wu Y, Xi J, Huo L, Padvorac J, **Shin EJ**, Giday SA, Lennon AM, Canto MI, Hwang JH, Li X. "Robust high-resolution fine OCT needle for side-viewing interstitial tissue imaging." IEEE Journal on Selected Topics in Quantum Electronics. 2010 Jul;16(4):863-869.

32. Dray X, Krishnamurty DM, Donatelli G, Gabrielson KL, Wroblewski RJ, **Shin EJ**, Giday SA, Buscaglia JM, Pipitone LJ, Marohn MR, Kalloo AN, Kantsevoy SV. "Gastric wall healing after NOTES procedures: closure with endoscopic clips provides superior histological outcome compared with threaded tags closure.' Gastrointest Endosc. 2010 Aug;72(2):343-350.
33. Lennon AM, Newman N, Makary MA, Edil BH, **Shin EJ**, Khashab MA, Hruban RH, Wolfgang CL, Schulick RD, Giday S, Canto MI. "EUS-guided tattooing before laparoscopic distal pancreatic resection (with video)." Gastrointest Endosc. 2010 Nov;72(5):1089-94.
34. Khashab MA, Yong E, Lennon AM, **Shin EJ**, Amateau S, Hruban RH, Olino K, Giday S, Fishman EK, Wolfgang CL, Edil BH, Makary M, Canto MI. "EUS is still superior to multidetector computerized tomography for detection of pancreatic neuroendocrine tumors." Gastrointest Endosc. 2010 Nov 8.
35. Khashab MA, Lennon AM, Singh VK, **Shin EJ**, Canto MI, Kalloo AN, Okolo PI 3rd, Giday SA. "EUS-guided pseudocyst drainage as a one-step procedure using a novel multiple wire insertion technique." Endoscopy. 2010;42 Suppl 2:E292-3.
36. Giday SA, Kim Y, Krishnamurty DM, Ducharme R, Liang DB, **Shin EJ**, Dray X, Hutcheon D, Moskowitz K, Donatelli G, Rueben D, Canto MI, Okolo PI, Kalloo AN. "Long-term randomized controlled trial of a novel nanopowder hemostatic agent (TC-325) for control of severe arterial upper gastrointestinal bleeding in a porcine model." Endoscopy. 2011 Apr;43(4):296-9. Epub 2011 Mar 7.
37. Khashab MA, **Shin EJ**, Amateau S, Canto MI, Hruban RH, Fishman EK, Cameron JL, Edil BH, Wolfgang CL, Schulick RD, Giday S. "Tumor Size and Location Correlate With Behavior of Pancreatic Serous Cystic Neoplasms." Am J Gastroenterol. 2011 Aug;106(8):1521-6.
38. Khashab MA, Hutfless S, Kim K, Lennon AM, Canto MI, Jagannath SB, Okolo PI 3rd, **Shin EJ**, Singh VK. "A Comparative Evaluation of Early Stent Occlusion Among Biliary Conventional Versus Wing Stents". Dig Dis Sci. 2012 Jun;57(6):1708-16. Epub 2012 Jan 20.
39. **Shin EJ**, Amateau SK, Kim Y, Gabrielson KL, Montgomery EA, Khashab MA, Chandrasekhara V, Rolshud D, Giday SA, Canto MI. "Dose-dependent depth of tissue injury with carbon dioxide cryotherapy in porcine GI tract." Gastrointest Endosc. 2012 May;75(5):1062-7. Epub 2012 Jan 31.
40. Magno P, Khashab MA, Mas M, Giday SA, Buscaglia JM, **Shin EJ**, Dray X, Kalloo AN. Natural orifice translumenal endoscopic surgery for anterior spinal procedures. Minim Invasive Surg. 2012;2012:365814. Epub 2012 May 24.
41. Agrawal N, Jiao Y, Bettegowda C, Hutfless SM, Wang Y, David S, Cheng Y, Twaddell WS, Latt NL, **Shin EJ**, Wang LD, Wang L, Yang W, Velculescu VE, Vogelstein B, Papadopoulos N, Kinzler KW, Meltzer SJ. "Comparative genomic analysis of esophageal adenocarcinoma and squamous cell carcinoma." Cancer Discov. 2012 Aug 16. [Epub ahead of print]
42. Gultepe E, Randhawa JS, Kadam S, Yamanaka S, Selaru FM, **Shin EJ**, Kalloo AN, Gracias DH. "Biopsy with Thermally-Responsive Untethered Microtools." Adv Mater. 2012 Oct 9. doi: 10.1002/adma.201203348. [Epub ahead of print]

43. Khashab MA, Kim KJ, Tryggestad EJ, Wild AT, Roland T, Singh VK, Lennon AM, **Shin EJ**, Ziegler MA, Sharaiha RZ, Canto MI, Herman JM. "Comparative analysis of traditional and coiled fiducials implanted during EUS for pancreatic cancer patients receiving stereotactic body radiation therapy." Gastrointest Endosc. 2012 Nov;76(5):962-71.
44. Khashab MA, Sharaiha RZ, Saxena P, Law JK, Singh VK, Lennon AM, **Shin EJ**, Canto MI, Aguila G, Okolo PI 3rd, Stavropoulos SN, Inoue H, Pasricha PJ, Kalloo AN. "Novel technique of auto-tunneling during peroral endoscopic myotomy (with video)." Gastrointest Endosc. 2013 Jan;77(1):119-22.
45. Khashab M, Alawad AS, **Shin EJ**, Kim K, Bourdel N, Singh VK, Lennon AM, Hutfless S, Sharaiha RZ, Amateau S, Okolo PI, Makary MA, Wolfgang C, Canto MI, Kalloo AN. "Enteral stenting versus gastrojejunostomy for palliation of malignant gastric outlet obstruction." Surg Endosc. 2013 Jan 9.
46. Gultepe E, Randhawa JS, Kadam S, Yamanaka S, Selaru FM, **Shin EJ**, Kalloo AN, Gracias DH. "Biopsy with thermally-responsive untethered microtools." Adv Mater. 2013 Jan 25;25(4):514-9.
47. Law JK, Singh VK, Khashab MA, Hruban RH, Canto MI, **Shin EJ**, Saxena P, Weiss MJ, Pawlik TM, Wolfgang CL, Lennon AM. "Endoscopic ultrasound (EUS)-guided fiducial placement allows localization of small neuroendocrine tumors during parenchymal-sparing pancreatic surgery." Surg Endosc. 2013 Apr 30.
48. Khashab MA, Kim K, Lennon AM, **Shin EJ**, Tignor AS, Amateau SK, Singh VK, Wolfgang CL, Hruban RH, Canto MI. "Should we do EUS/FNA on patients with pancreatic cysts? The incremental diagnostic yield of EUS over CT/MRI for prediction of cystic neoplasms." Pancreas. 2013 May;42(4):717-21.
49. Yang X, Song JH, Cheng Y, Wu W, Bhagat T, Yu Y, Abraham JM, Ibrahim S, Ravich W, Roland BC, Khashab M, Singh VK, **Shin EJ**, Yang X, Verma AK, Meltzer SJ, Mori Y. "Long non-coding RNA HNF1A-AS1 regulates proliferation and migration in oesophageal adenocarcinoma cells." Gut. 2013 Sep 2.
50. Sharaiha RZ, Kim KJ, Singh VK, Lennon AM, Amateau SK, **Shin EJ**, Canto MI, Kalloo AN, Khashab MA. "Endoscopic stenting for benign upper gastrointestinal strictures and leaks." Surg Endosc. 2013 Sep 7. [Epub ahead of print]
51. Kumbhari V, Gondal B, Okolo Iii PI, Lennon AM, Law JK, Singh VK, Saxena P, **Shin EJ**, Canto MI, Kalloo AN, Khashab MA. "Endoscopic ultrasound-guided angiotherapy of a large bleeding gastrointestinal stromal tumor." Endoscopy. 2013 Oct;45 Suppl 2:E326-7. doi: 10.1055/s-0033-1344875. Epub 2013 Oct 7.
52. Wild AT, Hiniker SM, Chang DT, Tran PT, Khashab MA, Limaye MR, Laheru DA, Le DT, Kumar R, Pai JS, Hargens B, Sharabi AB, **Shin EJ**, Zheng L, Pawlik TM, Wolfgang CL, Koong AC, Herman JM. "Re-irradiation with stereotactic body radiation therapy as a novel treatment option for isolated local recurrence of pancreatic cancer after multimodality therapy: experience from two institutions." J Gastrointest Oncol. 2013 Dec;4(4):343-51.
53. Li L, Masica D, Ishida M, Tomuleasa C, Umegaki S, Kalloo AN, Georgiades C, Singh VK, Khashab M, Amateau S, Li Z, Okolo P, Lennon AM, Saxena P, Geschwind JF, Schlachter T, Hong K, Pawlik TM, Canto M, Law J, Sharaiha R, Weiss CR,

Thuluvath P, Goggins M, **Shin EJ**, Peng H, Kumbhari V, Hutfless S, Zhou L, Mezey E, Meltzer SJ, Karchin R, Selaru FM. "Human bile contains microRNA-laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis." Hepatology. 2014 Feb 4. doi: 10.1002/hep.27050. [Epub ahead of print]

Book Chapters, Monographs

1. **Shin EJ**, Canto MI. The Clinical Assessment of Pancreatic Cancer. In: Cameron J, ed. Diseases of the Pancreas: Current Surgical Therapy.
2. **Shin EJ**. A Benign Form of Progressive Dysphagia. In: Kalloo AN and Buscaglia J, ed. Complicated Cases in GI.
3. **Shin EJ**. AIDS and Nodular Gastric Antritis. In: Kalloo AN and Buscaglia J, ed. Complicated Cases in GI.
4. **Shin EJ**. Twisted Turn of Events. In: Kalloo AN and Buscaglia J, ed. Complicated Cases in GI.
5. **Shin EJ**. A 76-Year-Old Male with Chronic Watery Diarrhea. In: Kalloo AN and Buscaglia J, ed. Complicated Cases in GI.
6. **Shin EJ**. Diarrhea, Abdominal Pain, and a Dilated Biliary Tree. In: Kalloo AN and Buscaglia J, ed. Complicated Cases in GI.
7. **Shin EJ**. Fulminant Hepatic Failure Following an Elective Colonoscopy. In: Kalloo AN and Buscaglia J, ed. Complicated Cases in GI.
8. **Shin EJ**, Giday SA. Endoscopic Techniques in Colorectal Neoplasia. In: Gearhart SL and Ahuja N, ed. Early Diagnosis and Treatment of Cancer: Colorectal Cancer.

Invited Reviews

1. **Shin EJ**, Kalloo AN. "Transcolonic NOTES: Current Experience and Potential Implications for Urologic Applications." J Endourol. 2009 Apr 30. [Epub ahead of print]
2. **Shin EJ,** Kalloo AN. "Endoscopic Transcolonic Techniques for NOTES." Techniques in Gastrointest Endosc. 2009 Apr:11(2):72-74.
3. Wu Y, Xi J, Huo L, Padvorac J, **Shin EJ**, Giday SA, Lennon AM, Canto MI, Hwang JH, Li X. "Robust Hihg-Resolution Fine OCT Needle for Side-Viewing Interstitial Tissue Imaging." IEEE Journal of Selected Topics in Quantum Electronics. 2010 July/Aug:16(4):863-869.
4. **Shin EJ**, Canto MI. "Pancreatic cancer screening." Gastroenterol Clin North Am. 2012 Mar;41(1):143-57. Epub 2012 Jan 5.
5. Zhang M, **Shin EJ**. "Successful endoscopic strategies for difficult polypectomy." Curr Opin Gastroenterol. 2013 Sep;29(5):489-894.
6. **Shin EJ**, Khashab M. "The role of endoscopy in the treatment, management, and personalization of pancreatic cancer." Curr Probl Cancer. 2013 Sep Oct; 37(5):293-300.

EXTRAMURAL FUNDING (CURRENT, PENDING, PREVIOUS)

2006-2008 NIH K12 Clinical Research Scholar
K12 HD049104-01
Principal Investigator: Neil Powe, M.D., M.P.H., M.B.A.
Role: Clinical Research Scholar

2010 Title: OCT Image-guided Biopsy Needle
NIH/NCI
R21 CA116442
Principal Investigator: Xingde Li
Role: Co-investigator

2010 Title: Molecular Contrast Agents Based on Gold Nanocages for OCT Imaging of Cancer
NIH/NCI
R01 CA120480-01
Principal Investigator: Xingde Li
Role: Co-investigator

2011 Title: Demonstration of an in vivo biopsy of the pancreatobiliary system surgical procedure usinG wireless miniaturized grippers
INBT Bio Medicine Pilot Projects Grant
90041725
Principal Investigators: Eun Ji Shin, MD; Anthony Kalloo, MD; David Gracias, PhD

2011-2015 Title: Nonlinear Optical Endomicroscopy for Optical Biopsy of Cancer in Internal Organs
NIH/NCI
R01 CA153023-01A1
Principal Investigator: Xingde Li
Role: Co-investigator

2012-2017 Title: Image-guided Bariatric Arterial Embolization (BAE) for the treatment of obesity
NIH/ NIBIB
R01DK097267-01
Principal Investigator: Kraitchman – Weiss
Role: Co-investigator

EDUCATIONAL ACTIVITIES

Teaching

2008 GI Pathophysiology Course small group for medical students

2010 Genes to Society GI Liver Course (Imaging of the GI Tract)

CLINICAL ACTIVITIES

Certification

2004 Internal Medicine

2008 Gastroenterology

Clinical (Service) Responsibilities

2008-present Endoscopy fellows coverage

2009-present Inpatient and consult service

ORGANIZATIONAL ACTIVITIES

Institutional Administrative Appointments

2010-present Director of Endoscopic Ultrasound program

2010-present Director of Advanced Endoscopy Fellowship Program

2011-present Endoscopy Safety Officer for GI

2013-present Co-Director of Gastroenterology Fellowship

Professional Societies

2005 American Gastroenterological Association

2005 American Society of Gastrointestinal Endoscopy
2007 Society of American Gastrointestinal and Endoscopic Surgeons
2008 American College of Gastroenterology

Conference Organizer, Session Chair:

2011 Course director, 10th Annual Gastroenterology Symposium for Nurses, Advanced Practice Nurses (NPs and CRNAs) and Physician Assistants

2012 Workshop director, 12th Annual Gastroenterology and Hepatology. Viva la Vida. Hands-On Workshop: Endoscopic Therapies for Barrett's Esophagus.

2013 Course director, 11th Annual Gastroenterology Symposium for Nurses, Advanced Practice Nurses (NPs and CRNAs) and Physician Assistants

RECOGNITION

Awards, Honors

2007 Clinical Researcher Award, Graduate Training Program in Clinical Investigation

1999-2000 Howard Hughes Medical Institute-National Institute of Health Research Scholar

1997 Phi Beta Kappa, Harvard University

1997 Summa Cum Laude, Harvard University

1996-1997 Harvard College Research Program Summer Grant

Talks, Conferences

2008 The Winning Concepts of Gastroenterology and Hepatology: The View of International Experts. "Mucosal Resection and Polyp Removal: Level the Playing Field."

2008 34th Annual Topics in Gastroenterology and Liver Disease: Medical and Surgical Aspects. "Obesity and GI Cancer Risk."

2009 9th Annual Gastroenterology and Hepatology. Viva la Vida. "Impact of NOTES on Therapeutic Endoscopy."

2009 35th Annual Topics in Gastroenterology and Liver Disease: Medical and Surgical Aspects. "Techniques for Endoscopic Mucosal Resection and Endoscopic Submucosal Dissection" and "Endoscopic Management of Variceal Bleeding: Band, Glue or Spray?"

2010 10th Annual Gastroenterology and Hepatology. Viva la Vida. "New Techniques for Colonoscopy" and "Endoscopic Therapy of Obesity"

2010 9th Annual Gastroenterology Symposium for Nurses, Nurse Practitioners and Physician Assistants. "Novel Treatments for GI Bleeding," "EMR and ESD" and "The Obesity Epidemic."

2010 ACG 2010 Hands-On Workshop Center "Mucosal Ablation" Faculty Leader and "Advanced Imaging" Faculty

2010 Chest 2010 "Natural Orifice Transluminal Endoscopic Surgery (NOTES): Past, Present, and Future"

2010 36th Annual Topics in Gastroenterology and Liver Disease: Medical and Surgical Aspects. "Submucosal Bumps in the Stomach: Which Ones Do We Care About?" and "New Indications for EUS"

2011 11th Annual Gastroenterology and Hepatology. Viva la Vida. "Imaging Modalities in Pancreatitis: What Are Available and What's Best," "How to do EMR and ESD in the Esophagus" and "Is Endoscopic Treatment of Obesity in Our Crystal Ball?"

2011 Innovations in Gastroenterology: A Three-Day Scientific Symposium Featuring Updates on Hepatology, Endoscopic Ultrasound (EUS) and NOTES. "Endoscopic Diagnosis of Pancreatic Cancer," "New Imaging

Techniques in Endoscopy," "New Techniques for Colonoscopy," "Techniques for Endoscopic Mucosal Resection and Endoscopic Submucosal Dissection"

2011 10th Annual Gastroenterology Symposium for Nurses, Nurse Practitioners and Physician Assistants. Course Director. "Modern Diagnosis of Occult GI Bleeding," "Current Spectrum of Endoscopic Procedures" and "When is Endoscopic Ultrasound Needed?"

2011 ACG 2011 Hands-On Workshop Center "Mucosal Ablation" Faculty Leader

2011 37th Annual Topics in Gastroenterology and Liver Disease: Medical and Surgical Aspects. "Endoscopic Management of Post-Bariatric Problems" and "Bariatric Endoscopy"

2012 29th Annual Medical & Surgical Gastroenterology: A Multidisciplinary Approach. "Surveillance and Ablative Techniques for Barrett's Esophagus", "My Patient has a Pancreatic Cyst: How Can I Help?" and "Hepatopancreaticobiliary Interactive Case Management"

2012 12th Annual Gastroenterology and Hepatology. Viva la Vida. "Gastric Cancer: Is There a Role for the Gastroenterologist?," "The Endoscopic Approach to Lower GI Bleeding" and "The Difficult Polypectomy"

2012 ACG 2012 Hands-On Workshop Center "Mucosal Ablation Methods" Faculty Leader

2012 38th Annual Topics in Gastroenterology and Hepato-Biliary Update Conference. "Management of Varices in 2012", "Bariatrics and the GI Endoscopist" and "Polypectomy: A Structured Approach."

2013 ACG 2013 Eastern Regional PG Course Hands-On Workshop Center "ERCP" Faculty

2013 13th Annual Gastroenterology and Hepatology. Viva la Vida. "Barrett's Esophagus: Who to Treat and How to Treat" and "Tips for Successful and Safe Polypectomy"

2013 11th Annual Gastroenterology Symposium for Nurses, Nurse Practitioners and Physician Assistants. Course Director. "Principles of Endoscopic management of GI Bleeding," "GI Bleeding Case-Based Interactive

Session," "The Difficult Polypectomy," and "Polypectomy Case-Based Interactive Session"

2013 International Society of Gastrointestinal Oncology 2013. "Screening for Pancreatic Cancer: Managing Uncertainty."

2013 39th Annual Topics in Gastroenterology and Hepato-Biliary Update Conference. "The Gut Microbiome and Cancer", "Roundtable Discussions with Faculty- Endoscopy and the Bariatric Patient", "What to Do with That "Difficult" Polyp", and "Endoscopy in the High Risk Patient."

OTHER PROFESSIONAL ACCOMPLISHMENTS

Abstracts at National Meetings

1. Giday SA, Ko C, Clarke JO, **Shin EJ**, Magno P, Jagannath SB, Canto MI, Buscaglia JM, Kansevoy SV. Endoscopic Ultrasound (EUS)-Guided Portal Vein CO2 Angiography: A Pilot Study in a Porcine Model. Gastroenterology, April 2007.
2. GIday SA, Clarke JO, Buscaglia JM, **Shin EJ**, Jagannath SB, Canto MI, Ko C, Magno P, Kantsevoy SV. Endoscopic Ultrasound (EUS)-Guided Portal Vein Catherization: A Novel Promising Approach for Portal Angiography and Portal Vein Pressure Measurements. Gastroenterology, April 2007.
3. Magno P, Giday SA, Gabrielson KL, **Shin EJ**, Buscaglia JM, Clarke JO, Ko C, Jagannath SB, Canto MI, Sedrakyan G, Kantsevoy SV. Endoscopic Ultrasound (EUS)-Guided Submucosal Implantation of a Radio-Opaque Marker: A Simple and Effective Procedure to Facilitate Subsequent Surgical and Radiation Therapy. Gastroenterology, April 2007.
4. Magno P, Giday SA, Gabrielson KL, **Shin EJ**, Buscaglia JM, Clarke JO, Ko CW, Jagannath SB, Canto MI, Sedrakyan G, Kantsevoy SV. Endoscopic Ultrasound (EUS)-Guided Implantation of a Radio-Opaque Marker into Mediastinal and Retroperitoneal Lymph Nodes is Safe and Effective. Gastroenterology, April 2007.
5. Clarke JO, **Shin EJ**, Magno P, Giday SA, Buscaglia JM, Sedrakyan G, Kalloo AN, Kantsevoy SV. A Novel Implantable On-Demand Microstimulator Can Increase Resting Anal Sphincter Pressure: A Pilot Study in a Porcine Model. Gastroenterology, April 2007.
6. Ko C, **Shin EJ**, Magno P, Giday SA, Buscaglia JM, Clarke JO, Jagannath SB, Chung S, Cotton PB, Gostout CJ, Hawes RH, Pasrichar PJ, Kalloo AN, Kantsevoy SV. Preliminary Pneumoperitoneum Facilitates Transgastric Access Into the Peritoneal Cavity for NOTES. Gastroenterology, April 2007.

7. Buscaglia JM, **Shin EJ**, Clarke JO, Giday SA, Ko C, Thuluvath PJ, Isakovich NV, Sedrakyan G, Magno P, Kantsevoy SV. Endoscopic Retrograde Cholangiopancreatography (ERCP), but not EGD or Colonoscopy, Significantly Increases Portal Venous Pressure: Direct Portal Pressure Measurements Through EUS-Guided Catherization. Gastroenterology, April 2007.
8. Clarke JO, Giday SA, Magno P, Buscaglia JM, **Shin EJ**, Jagannath SB, Mullin GE. How Good Is Capsule Endoscopy For Detection of Periampullary Lesions? Results of a Tertiary Referral Center. Gastroenterology, April 2007.
9. Giday SA, Dray X, **Shin EJ**, Buscaglia JM, Wroblewski RJ, Lyn-Sue J, Magno P, Marohn MR, Kantsevoy SV, Kalloo AN. NOTES Is a Highly Effective Technique for the Evaluation of Acute Penetrating Abdominal Injury. Gastroenterology, April 2008.
10. Giday SA, Buscaglia JM, Althaus J, Dray X, **Shin EJ**, Ruben D, Wroblewski RJ, Kantsevoy SV, Kalloo AN. Successful Diagnostic and Therapeutic Intrauterine Fetal Interventions By NOTES. Gastroenterology, April 2008.
11. Buscaglia JM, Kapoor S, **Shin EJ**, Okolo P. Factors Associated with Hospital Death in Patients Admitted with Pancreatitis. Gastroenterology, April 2008.
12. Dray X, Li Z, Hua J, Giday SA, Redding SK, **Shin EJ**, Wroblewski RJ, Buscaglia JM, Magno P, Ruben D, Schweitzer MA, Clark J, Kalloo AN. Removal of Visceral Fat Improves Metabolic Syndrome and Hepatic Steatosis in Diet-Induced Obese Mice. Gastroenterology, April 2008.
13. Kantsevoy SV, Dray X, **Shin EJ**, Buscaglia JM, Magno P, Chung SCS, Cotton P, Gostout CJ, Hawes RH, Kalloo AN, Pasricha PJ, Assumpcao LR, Marohn MR, Redan JA, Giday SA. Transgastric Ventral Hernia Repair: A Randomized Controlled Study in a Live Porcine Model. Gastroenterology, April 2008.
14. Giday SA, Wroblewski RJ, Magno P, Buscaglia JM, **Shin EJ**, Dray X, Lyn-Sue J, Marohn MR, Kantsevoy SV, Kalloo AN. Novel Endoscopic Techniques Are Effective for Control of Hemorrhage During NOTES. Gastroenterology, April 2008.
15. Magno P, Mas MA, Rivera Y, Giday SA, Buscaglia JM, **Shin EJ**, Kantsevoy SV, Dray X, Kalloo AN. NOTES Is Successful for Vertebral Spinal Interventions with Significant Advantages for Anterior Spinal Procedures. Gastroenterology, April 2008.
16. Dray X, Giday SA, Buscaglia JM, Redding SK, Wroblewski RJ, Assumpcao LR, **Shin EJ**, Magno P, Pipitone LJ, Marohn MR, Kalloo AN, Kantsevoy SV. The Hydrogen-Based Quantitative Leak Test Is Rapid, Minimally Invasive and Highly Accurate for Closure Assessment After NOTES Procedures. Gastroenterology, April 2008.
17. Giday SA, **Shin EJ**, Kantsevoy SV. Autologous Blood Provides the Best Cushioning Effect for Polypectomy: A Prospective Randomized Controlled Human Trial. Gastroenterology, April 2008.
18. Dray X, Gabrielson K, Buscaglia JM, **Shin EJ**, Giday SA, Surti VC, Assumpcao LR, Marohn MR, Magno P, Pipitone LJ, Redding SK, Kantsevoy SV. Air and Fluid Leak Tests Post NOTES Procedures: A Pilot Study in a Live Porcine Model. Gastroenterology, April 2008.

19. Buscaglia JM, Simons B, Prosser BJ, Ruben D, Giday SA, Magno P, Clarke JO, **Shin EJ**, Kalloo AN, Gabrielson K, Jagannath SB. Severity of Post-ERCP Pancreatitis Directly Proportional to the Invasiveness of Endoscopic Intervention: A Pilot Study in a Canine Model. Gastroenterology, April 2008.
20. Conde JG, Vega A, Magno P, Más M, Sierra R, Shipley R, Kuhn E, Rudich D, Frankford L, Clarke J, Buscaglia J, **Shin EJ**, Levitz D, Canto M, Kalloo AN. Use of DVTS for Live Transmission of Medical Endoscopic Procedures. Paper presented at: ResearchChannel/Internet2 Working Group. Spring 2008 Internet2 Member Meeting; 2008.
21. Conde JG, Vega A, Magno P, Más M, Sierra R, Shipley R, Kuhn E, Rudich D, Frankford L, Clarke J, Buscaglia J, **Shin EJ**, Levitz D, Canto M, Kalloo AN. Use of DVTS for Live Transmission of Medical Endoscopic Procedures. Paper presented at: Collaborations Special Interest Group. Spring 2008 Internet2 Member Meeting; 2008.
22. Assumpcao L, Dray X, Donatelli G, Giday SA, Mukkai Krishnamurty D, Buscaglia JM, **Shin EJ**, Pipitone L, Marohn MR, Kantsevoy SV. Direct Comparison of Different Techniques of Transgastric Access for NOTES Procedures. 16th United European Gastroenterology Week 2008.
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